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The Seed Ecology of Rare and Endangered Gibbens' Beardtongue (*Penstemon gibbensii*) and Blowout Penstemon (*Penstemon haydenii*)

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The Seed Ecology of Rare and Endangered Gibbens' Beardtongue (*Penstemon gibbensii*)
and Blowout Penstemon (*Penstemon haydenii*)

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A thesis submitted to the faculty of
Brigham Young University
in partial fulfillment of the requirements for the degree of
Master of Science

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ABSTRACT

The Seed Ecology of Rare and Endangered Gibbens' Beardtongue (*Penstemon gibbensii*) and Blowout Penstemon (*Penstemon haydenii*)

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Penstemon gibbensii and *Penstemon haydenii* are two rare, perennial forbs inhabiting remote areas of the western United States. *P. gibbensii* is listed as a sensitive species by the Bureau of Land Management (BLM) in Colorado, Utah, and Wyoming (Heidel, 2009). *P. haydenii* was designated as Endangered by the U.S. Fish and Wildlife Service in 1987 (Heidel, 2012). This thesis research was geared toward helping land managers in their efforts to protect and rehabilitate these species by providing understanding on different aspects of their seed ecology.

My first study was a laboratory experiment performed on *P. gibbensii* and *P. haydenii* seed germination response to moist chilling and dry after-ripening. Wild harvested seeds were subjected to moist chilling at 2-4 °C for 0, 4, 8, 12, and 16 weeks and held in dry storage for approximately 2 years to determine effective methods for breaking primary dormancy. *P. gibbensii* seed germination increased consistently with increased length of chilling up to 16 weeks and exhibited habitat-correlated variation in this response. *P. haydenii* seed germination increased from 1 to 100% germination with 4 weeks of chilling. *P. haydenii* germination was greatest (96%) when incubated under a cool, diurnally-fluctuating temperature regime (10-20 °C) and responded positively to dry storage, increasing germination from 0 to 15%.

My second study was an *in situ* field study designed to characterize the active seed bank of *P. haydenii*. We set up a transect line across a *P. haydenii* population and measured the number of seeds entering the seed bank, lost to predation post-dispersal, and persisting in the seed bank. *P. haydenii* does not appear to form an ecologically significant seed bank. Approximately 140 seeds/ 10m² could potentially enter the seed bank but only 1 seed in the upper 10cm of sand persisted. Heavy post-dispersal insect predation resulted in a decrease in viability of nearly 30% in exposed *P. haydenii* seeds after just 12 hours.

My third study explored the effects of burial by sand on *P. haydenii*. Wild-harvested seeds were planted in pots at 1, 2, 4, 6, 8, and 10cm deep in sand and incubated at 10-20 °C. Seed germination and mortality and seedling emergence were measured. The response of dormant seeds to post-burial incubation was determined. Burial depth decreased seedling emergence and seed germination. Shallow burial appears to induce secondary dormancy for seeds that don't germinate quickly, whereas deep burial appears to impose enforced dormancy in burial.

Keywords: after-ripening, enforced dormancy, moist chilling, persistent seed bank, psammophyte, sand burial, secondary dormancy, seed germination

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CHAPTER 1

Breaking Seed Dormancy in Gibbens' Beardtongue (*Penstemon gibbensii*) and Blowout Penstemon (*Penstemon haydenii*): Germination Response to Moist Chilling and Dry After-ripening

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ABSTRACT- ARTICLE 1

This study explored the germination ecology of two rare, perennial forb species of the western United States, Gibben's beardtongue (*Penstemon haydenii* (Dorn) [Scrophulariaceae]) and blowout penstemon (*Penstemon haydenii* (Watson) [Scrophulariaceae]). Wild-harvested seeds were subjected to moist chilling at 2-4 °C for 0, 4, 8, 12, and 16 weeks and approximately 2 years of dry storage under laboratory conditions to determine effective methods for breaking primary dormancy. Seeds of both species responded positively to moist chilling. *Penstemon gibbensii* seed germination increased consistently with increasing length of chilling and exhibited habitat-correlated variation in this response. *Penstemon haydenii* seed germination increased from 1 to 100% germination with 4 weeks of chilling and remained high (> 95%) under favorable incubation regimes for all other chilling lengths. *Penstemon haydenii* germination was greatest (96%) when incubated under a cool, diurnally-fluctuating temperature regime (10-20 °C). Under warmer or constant temperatures post-chilling germination was < 20%. *Penstemon haydenii* seeds also exhibited an after-ripening response; dry storage for approximately 2 years under laboratory conditions increased germination without chilling from 0 to 15%. Our findings should prove useful in propagation and reintroduction efforts for restoring populations of these rare species.

INTRODUCTION

The regulation of seed germination timing has two main functions: (1) to optimize within-year germination timing for maximum seedling establishment success and (2) to prevent complete germination within a year and ensure carry-over of seeds (Meyer, 1992). The first function enables a species to prevent precocious germination under unfavorable environmental conditions. This appears to have evolved in response to selection regimes, particularly climate regimes, of distinct habitats (Pendleton, 2004) and is known as “predictive” dormancy (Venable and Lawlor, 1980). Predictive dormancy can often be broken by specific environmental cues that occur prior to optimal conditions for establishment (Venable and Lawlor, 1980). In the genus *Penstemon* this environmental cue is often the duration of moist chilling, which under natural conditions occurs beneath winter snowpack (Meyer, Kitchen and Carlson, 1995). For example, dormant seeds from multiple populations of *Penstemon* species responded differently in laboratory experiments imitating the duration of winter snowpack. Seeds from populations with more severe winters and greater snowpack required longer moist chilling periods than seeds from populations experiencing shorter, milder winters. The outcomes from common garden experiments corroborated with these results, suggesting a genetic basis for germination differences both among populations and between individual *Penstemon* plants (Meyer, Kitchen and Carlson, 1995).

The second function of seed germination regulation enables a species to establish a persistent seed bank. This regulation is referred to as “innate” dormancy and is not overcome by cues associated with optimum conditions for germination within the first year (Venable and Lawlor, 1980). High innate dormancy provides species with a hedge against complete germination under potentially unfavorable environmental conditions for seedling establishment

(Venable and Lawlor, 1980). Laboratory experiments performed on 16 *Penstemon* species revealed that many of these species produced a fraction of seeds exhibiting innate dormancy. These seeds did not respond to chilling the 1st year despite prolonged chilling (Kitchen and Meyer, 1991).

Penstemon gibbensii (Dorn) [Scrophulariaceae] and *Penstemon haydenii* (Watson) [Scrophulariaceae] are two rare perennial forbs. *Penstemon gibbensii* inhabits shale and sandy-clay slopes of the Brown's Park Formation in areas of Colorado, Utah, and Wyoming of the Western United States (Heidel, 2009) and very little is known concerning the germination ecology of this species. *P. haydenii* is endemic to the sand hills of west-central Nebraska and the Ferris Dunes of Carbon County, Wyoming (Heidel, 2012). It was listed as Endangered by the U.S. Fish and Wildlife Service in 1987 (Heidel, 2012) and extensive research has been performed on the Nebraska populations of this species. Flessner and Stubbendieck (1989) examined the effects of moist chilling at 3 °C on *P. haydenii* seeds and found that stratification for 6, 12, and 18 weeks enhanced germination from 8% to 21% on average, but concluded that it “did not consistently enhance germination.” However, the post-chilling incubation temperatures used in their study were much higher than the optimum temperatures reported for *Penstemon* germination (Allen and Meyer, 1990).

This study was conducted to better understand requirements for breaking primary seed dormancy in these two species. Our specific objectives were to 1) determine the role of moist chilling in breaking the primary dormancy of wild-collected *P. gibbensii* and *P. haydenii* seeds, 2) determine optimum post-chilling incubation temperatures for stimulating germination in *P. haydenii*, and 3) determine if wild-collected *P. gibbensii* and *P. haydenii* seeds exhibit an after-ripening response (i.e. increased germination following dry storage). Characterizing the

mechanisms regulating germination in these two rare *Penstemon* species may assist managers in their conservation efforts by 1) helping optimize propagation techniques for reintroductions and recovery efforts and 2) increasing their understanding of how these plants can be expected to respond to varying environmental conditions in the field. We hypothesized that 1) *P. gibbensii* and *P. haydenii* germination would increase with increased chilling length, 2) *P. gibbensii* germination in chill would be greater for lower elevation populations than higher elevation populations in prolonged chilling (> 8 weeks) (testable because we had multiple collections), 3) *P. haydenii* would favor cooler incubation temperatures, and 4) *P. gibbensii* and *P. haydenii* would exhibit an after-ripening response following prolonged dry storage.

MATERIALS AND METHODS

Seeds used in our experiments were collected from wild populations of *P. haydenii* and *P. gibbensii*. *Penstemon haydenii* seeds were collected from a single site located on Bear Mountain in the Ferris Dunes of Carbon Co., Wyoming. One seed lot was collected in the late summer of 2010 and the second was collected in the summer of 2012 (Table 1). Both seed collections were included in our experiments to determine the effects of time spent in dry storage for this species as well. *P. gibbensii* seeds were collected in the early fall of 2010 from three collection sites: Flat Top, Wyoming; Sand Creek, Wyoming and Brown's Park, Utah (Table 1). An additional collection made from the Brown's Park, Utah site in the fall of 2009 was also included in our experiments to examine the effects of time spent in dry storage on germination response.

Following collection, all seeds were cleaned by a process of screening and blowing and stored in manila envelopes under laboratory conditions (20-22 °C, 6-8% moisture content) until laboratory experiments were conducted. Initial viability for each seed lot was also determined using either a cut test (Association of Official Seed Analysts, 1988) or tetrazolium staining (Grabe, 1970).

Seeds were then subjected to a series of laboratory germination experiments. In December 2010 – March 2011 we determined if *P. gibbensii* exhibited primary dormancy and examined its response to moist chilling. Seeds from each of the four wild collections (Flat Top, WY; Sand Creek, WY; Brown's Park, UT 2010; and Brown's Park, UT 2009) were either placed directly into incubation at 10-20 °C (12 hour photoperiod corresponding to the high temperature) without a chilling pretreatment or subjected to one of four chilling treatments: 4, 8, 12 and 16

weeks of moist chilling in a dark chamber held at 2-4 °C. Following chill, dishes were placed in incubation at 10-20 °C (12 hour photoperiod) and scored for germination twice weekly.

In August 2012 - January 2013 *P. haydenii* primary dormancy and its response to moist chilling were studied as described for *P. gibbensii*, using seeds from each of the two *P. haydenii* seed lots (Bear Mountain 2010 and 2012).

To determine the optimum incubation temperatures required for *P. haydenii* germination, we put seeds from the Bear Mountain 2012 seed lot into moist chilling at 2-4 °C in a dark chamber for 4 weeks. Following chill, seeds were then subjected to one of six incubation treatments: constant 15 °C, alternating 10-20 °C, constant 20 °C, alternating 15-25 °C, constant 25 °C, or alternating 20-30 °C (12 hour photoperiod for all treatments). Dishes were scored for germination every two days for 4 weeks and ungerminated seeds were tested for viability at the end of the incubation period.

In the winter of 2010 *Penstemon gibbensii* and *P. haydenii* response to after-ripening was also determined. Seeds of *P. haydenii* and *P. gibbensii* were imbibed and placed directly into 10-20 °C incubation and scored for germination. Seeds from the same collections were stored for approximately 2 years under laboratory conditions (20-22 °C, 6-8% moisture content) in manila envelopes then once again imbibed and placed into 10-20 °C incubation and scored for germination. Germination data was compared for “recently harvested” and “stored” seeds to determine the after-ripening response.

All experiments included four replications of 25 seeds for each seed lot. Seeds were placed on water-saturated germination blotters (Anchor Paper, St. Paul, Minnesota) in 15 X 100 mm plastic Petri dishes to maintain adequate moisture. During incubation, dishes were read periodically and germinated seeds (radicle >1 mm) were counted and removed. At the conclusion

of each experiment, viability of ungerminated seeds was determined using either a cut test or tetrazolium staining. Viable, ungerminated seeds were classified as dormant.

Germination data from each experiment was converted to a proportion basis. Germination proportions were arcsine-transformed and analyzed using General Linear Models (GLM) to determine significant differences within and between treatments and collections. Differences in the means were determined using LSMEAN separations. All data were analyzed in SAS v. 8.1 (PROC GLM: SAS Institute, 2000).

RESULTS

***P. gibbensii* and *P. haydenii* primary dormancy:**

Recently harvested *P. gibbensii* seeds germinated to a maximum of 25% (percentages= proportions x 100) when placed directly into incubation without a chilling pretreatment (Figure 1). Seeds from Brown's Park, Utah (2010) germinated to 22%, Sand Point to 25%, and Flat Top, the population from the highest elevation, completely failed to germinate (Figure 1). There was a significant population effect on the proportion of seeds germinating in incubation without a chilling pretreatment. The Flat Top population yielded lower germination in incubation than the other two *P. gibbensii* populations ($P < 0.0001$) (Figure 1). Recently harvested (2012) *P. haydenii* seeds from Bear Mountain also failed to germinate; germination was only 1% without a chilling pretreatment (Figure 2).

***P. gibbensii* and *P. haydenii* response to incremental chilling:**

Seed germination for *P. gibbensii* increased consistently with the length of moist chilling ($P < 0.0001$) with 0 week chill having the lowest proportion of total seeds germinating and 16 weeks the highest (Figure 1). There was also a significant population effect on the total proportion of seeds germinating. Flat Top seeds germinated to a much lower percentage than all other collections ($p < 0.0001$). The Brown's Park 2010 seed collection had the highest total germination but this was not significantly different from the Sand Point population ($p = 0.0826$) (Figure 1). Chilling length had a significant treatment effect on the proportion of seeds germinating in chill ($P < 0.0001$). As chilling length increased, so did the proportion of seeds germinating in chill (Figure 1). Results also included a significant population effect on the

proportion of seeds germinating in chill. Flat Top yielded lower germination in chill than all other *P. gibbensii* seed collections ($P < 0.0001$) (Figure 1).

Chilling length also had a treatment effect on the total proportion of *P. haydenii* seeds germinating in incubation ($P < 0.0001$). Germination was lower for 0 weeks of chilling than for all other chilling treatment lengths (Figure 2). In contrast with *P. gibbensii*, following 4 weeks of chill the proportion of *P. haydenii* seeds germinating did start to decrease with increased chilling length; seeds chilled for 16 weeks had significantly lower germination than did those chilled for 4 or 8 week (Figure 2). There was a significant treatment by seed collection year effect as well. The 2012 Bear Mountain collection had lower germination with 0 weeks of chilling than the 2010 Bear Mountain collection ($P < 0.0001$) (Figure 2). Unlike *P. gibbensii*, *P. haydenii* seeds did not germinate in chill.

P. haydenii optimum incubation temperature:

Fluctuating incubation temperatures stimulated greater germination than did constant incubation temperatures ($p > 0.0001$). Germination percentages at all fluctuating temperatures were significantly greater than at any constant temperature (Figure 3). The 10-20 °C incubation treatment stimulated higher germination of *P. haydenii* seeds than any other incubation treatment ($P < 0.0001$) (Figure 3). Pre-chilled *P. haydenii* seeds placed in 10-20 °C incubation showed almost complete germination (Figure 3).

P. gibbensii and P. haydenii response to after-ripening:

Prolonged dry storage under laboratory conditions had no effect on the germination of

P. gibbensii seeds for any population ($P= 0.9647$). Brown's Park and Flat Top germination percentages increased slightly following approximately 2 years of prolonged dry storage but these numbers were not significantly different, and Sand Point seed germination actually decreased following dry storage (Figure 4). In contrast, *P. haydenii* seed germination was improved by after-ripening ($P < 0.0001$). In 2011 *P. haydenii* seeds collected the previous summer failed to germinate in incubation, whereas in 2012 the germination of seeds from the same collection increased significantly (Figure 4).

DISCUSSION

Our results indicate that seeds of both *P. gibbensii* and *P. haydenii* exhibit significant primary dormancy. Without any dormancy-breaking treatment neither species achieved high germination. For spring emerging species such as these, primary dormancy acts to prevent precocious germination under winter snowpack, while stimulating germination to follow shortly after snowmelt to maximize seedling establishment (Meyer and Kitchen, 1994). It is interesting to note that the extent of this primary dormancy response in *P. gibbensii* appears to vary with population elevation as hypothesized. Flat Top, the highest elevation *P. gibbensii* population in our study, had significantly lower germination percentages in incubation with or without a chilling pretreatment than all other populations. Increases in elevation have been shown to be associated with increased dormancy in the seeds of some alpine species (Cavieres and Arroyo, 2001). It is possible that selection pressures at higher elevations have favored greater levels of innate dormancy in *P. gibbensii* seeds.

Penstemon gibbensii and *P. haydenii* seeds both exhibited significant increases in germination following moist chilling. Moist chilling imitates conditions experienced under snowpack during winter. Snowpack keeps seeds moist and insulates them from freezing temperatures at the soil surface. Seeds that require a period of moist chilling prior to germination use winter duration as the environmental cue for breaking primary dormancy and are able to time radicle emergence to correspond with the arrival of spring (Meyer, Kitchen and Carlson, 1995). *Penstemon gibbensii* and *P. haydenii* both possess a chilling requirement for breaking primary dormancy. It is likely that the mechanism regulating primary dormancy for these species in the field is the duration of winter snowpack in their respective habitats.

Penstemon gibbensii seed germination in chill also increased with increased chilling length. Most *P. gibbensii* seeds from the lower elevation populations germinated in chill within 4 months. High levels of germination during moist chilling in the field would not likely happen. Precocious germination prior to spring snowmelt would likely result in the death of emerged seedlings due to frost and high levels of precocious germination would be selected against. It is unlikely then, that these populations of *P. gibbensii* ever experience winter snowpack for this prolonged length of time in the field. The Flat Top population resides at a much higher elevation with greater mean January precipitation, most likely in the form of snow, and exhibited significantly less germination in chill than the other two populations. According to Allen and Meyer (1998), the seeds of species that establish in the spring show variation among populations that can be associated with habitat conditions. According to our results, *P. gibbensii*, like many other Intermountain *Penstemon* species, appears to exhibit habitat correlated variation in germination response to moist chilling.

Penstemon haydenii seeds germinated almost completely following just 1 month of chilling. Unlike *P. gibbensii*, *P. haydenii* seed germination did not increase with increased chilling length beyond 4 weeks and no *P. haydenii* seeds germinated in chill. As a sand dune endemic, *P. haydenii* inhabits environments that are very dry much of the year with relatively small windows for timing successful seedling establishment. Seeds must therefore be able to accurately sense changes in weather from winter to spring and respond quickly enough to take advantage of available water before the sand dries out. This population of *P. haydenii* is also from a high elevation with long, severe winters. Germination during chill could result in seedling death from frost and we would expect for this population of *P. haydenii* to have tightly regulated primary dormancy so as to not germinate precociously. In the field *P. haydenii* does not form a

persistent seed bank (unpublished data). Reliance on current year seedling survival could potentially provide a selection pressure for more tightly regulated primary dormancy to prevent seedling death as a result of precocious germination.

Consistent with our hypothesis, *P. haydenii* seeds germinated best at cool, diurnally fluctuating incubation temperatures following moist chilling. When combined with at least one month of moist chilling, incubation at 10-20 °C stimulated much higher germination percentages than those reported by Flessner and Stubbendieck (1989), who combined moist chilling with higher post-chill incubation temperatures. When paired with the same warmer incubation temperatures, one month of moist chilling stimulated similarly low levels of germination as those achieved in their study. Previously assumed ineffective moist chilling appears to actually be a highly effective method for breaking primary dormancy and stimulating germination in *P. haydenii* seeds. It is possible that the drier, high elevation habitat in Wyoming causes populations of *P. haydenii* to respond differently than those from the lower elevation, more mesic habitats of their Nebraska congeners as a result of ecotypic variation. *Penstemon* germination responses to moist chilling and incubation have been shown to exhibit ecotypic variation associated with elevation and climate regimes (Meyer, Kitchen and Carlson, 1995; Meyer and Kitchen, 1994; Meyer, 1992). However, work performed on temperature requirements for *Penstemon* germination showed a marked decrease in germination at temperatures above 20 °C for three species tested (Allen and Meyer, 1990).

Penstemon haydenii also exhibited a preference for fluctuating incubation temperatures over constant ones. All variable incubation treatments yielded significantly higher germination than any of the constant incubation treatments. This positive seed response of *P. haydenii* to fluctuating temperatures may be an indicator of its ability sense seed burial. This response to

temperature fluctuation has often been associated with a seed's ability to sense and respond to diurnal temperature changes near the soil surface, which would stimulate germination with shallow burial and inhibit it with deep burial as reviewed by Thompson (1974). Soil acts as a buffer against temperature variation and fluctuations in temperature are dampened as soil depth increases (Ghersa, 1992). As a sand dune endemic, burial by sand is a frequent occurrence for *P. haydenii* seeds. The ability to restrict germination to optimum burial depths would be beneficial to seedling success. It is likely that *P. haydenii*'s positive response to fluctuating temperatures acts as a depth-sensing mechanism to prevent germination of deeply buried seeds and stimulate germination of shallowly buried seeds.

After-ripening, the processes of breaking primary dormancy through dry storage (Hilhorst and Karssen, 1992), did not induce germination of *P. gibbensii* seeds. Similar results were reported for *Penstemon eatonii*, where it was concluded that environmental cues received in the imbibed state were more important for stimulating seed germination than length of storage in a dry state (Meyer, 1992). It is likely that *P. gibbensii* also relies more heavily on environmental cues received in the imbibed state for germination than after-ripening of its seeds. *Penstemon haydenii*, however, responded as predicted and showed a positive response to prolonged dry storage. Selection pressures in different habitats can result in seed adaptations specifically tailored to that environment (Beckstead, Meyer, and Allen, 1996). One such adaptation is a change in primary seed dormancy during after-ripening (Beckstead, Meyer and Allen, 1996). *Penstemon haydenii* seeds may experience increased mortality in the soil through time and lose dormancy in response to prolonged dry storage because the odds of surviving precocious germination are better than the odds of surviving longer in the soil. However, the likelihood of experiencing a moist chilling period of at least one month during winter is high and it is probable

that few seeds buried shallowly in the soil fail to germinate the first spring following dispersal. This dry after-ripening response may be more useful to conservationists planning on propagating *P. haydenii* because it indicates that if the seeds are left in dry storage for long enough they may not need a chilling treatment to stimulate germination.

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TABLE AND FIGURE CAPTIONS

Table 1: Location and climate data for *P. gibbensii* and *P. haydenii* seed collections. Mean January temperature and precipitation data for each site were generated using the Prism database (www.prism.oregonstate.edu).

Figure 1: Proportions of *P. gibbensii* seeds germinated during and following chilling as a function of chilling duration for each seed collection. (A) Brown's Park, UT 2009; (B) Brown's Park, UT 2010; (C) Sand Point, WY; (D) Flat Top, WY. Columns with different letters represent treatments with significant differences ($P < 0.05$) in total proportion of seeds germinated as determined in the GLM means separations.

Figure 2: The proportion of Bear Mountain *P. haydenii* seeds germinating (\pm SE) during incubation (10-20 °C) following different lengths of moist chilling (2-4 °C) for each seed collection. (A) Bear Mountain 2010; (B) Bear Mountain 2012.

Figure 3: The proportion of *P. haydenii* seeds (\pm SE) germinating under constant or fluctuating incubation temperatures following 4 weeks of moist chilling (2-4 °C).

Figure 4: The proportion of 2010 collections of *P. haydenii* and *P. gibbensii* (\pm SE) germinating during incubation (10-20 °C) following 4 weeks of moist chilling (2-4 °C) when recently harvested (2010) and after two years of dry storage (2012).

TABLES AND FIGURES

Table 1

Species	Site	Location	Elevation (m)	Mean Jan Temp. (°C)	Mean Jan. Precip.	Viability (%)
<i>P. gibbensii</i>	Utah Brown's Park (2009)	Daggett Co., UT	1700	-4.6	10.0mm	82
<i>P. gibbensii</i>	Utah Brown's Park (2010)	Daggett Co., UT				92
<i>P. gibbensii</i>	Sand Creek	Carbon Co., WY	1890-1900	-7	20.2mm	96
<i>P. gibbensii</i>	Flat Top	Carbon Co., WY	2290-2350	-5.9	33.3mm	80
<i>P. haydenii</i>	Bear Mountain (2010)	Carbon Co., WY	2070-2100	-5.9	15.2mm	93
<i>P. haydenii</i>	Bear Mountain (2012)	Carbon Co., WY				97

Figure 1

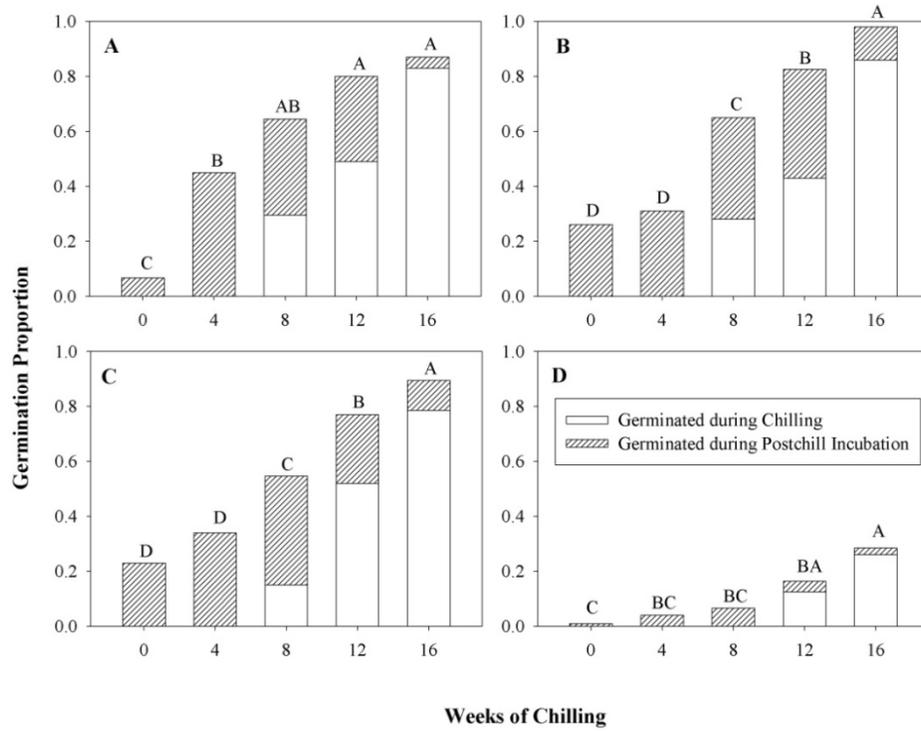


Figure 2

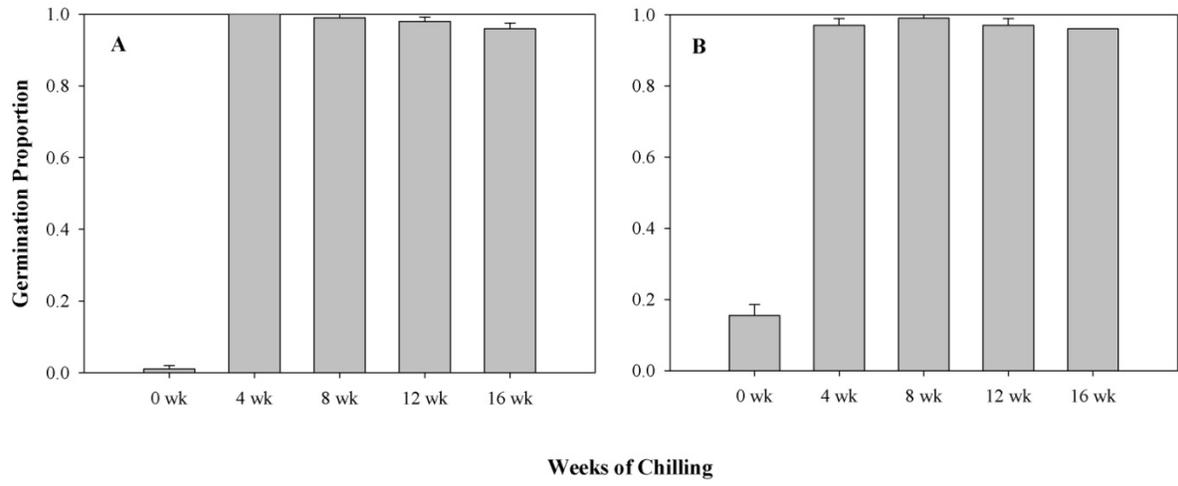


Figure 3

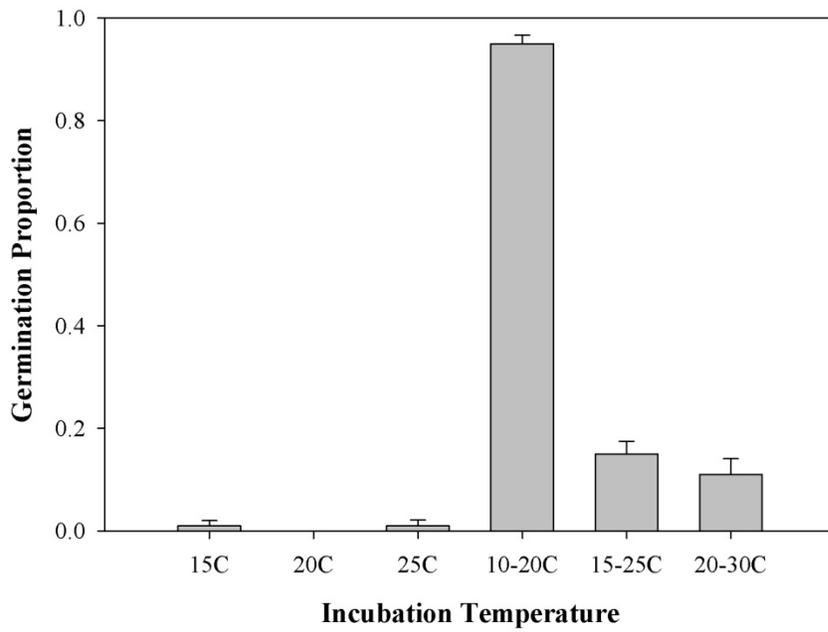
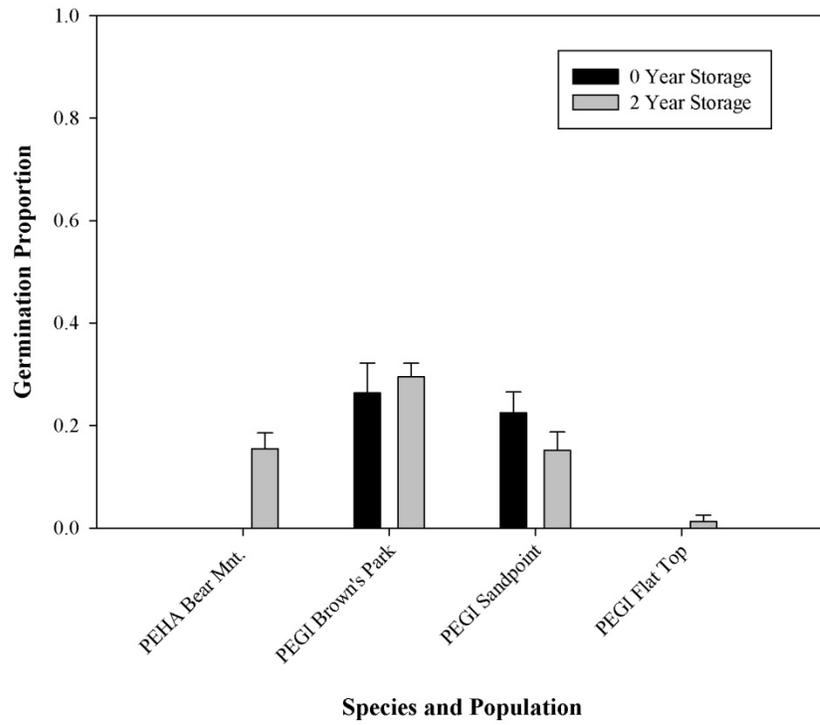


Figure 4



CHAPTER 2

Characteristics of the Shallow Seed Bank of the Sand Dune Endemic Blowout Penstemon (*Penstemon haydenii*) in Wyoming

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ABSTRACT- ARTICLE 2

The formation of a persistent soil seed bank can be critical to the survival of species inhabiting sand dune systems. Blowout Penstemon (*Penstemon haydenii* Wats. Scrophulariaceae) is an early colonizer of active sand dunes and this study was conducted to characterize the seed bank ecology of this endangered species. To do this we set up a transect line across a *P. haydenii* population and estimated seed production, post-dispersal predation, and seed persistence in the shallow seed bank (0-10 cm depth) in relation to plant density and sand dune movement. Our results showed that approximately 140 seeds/ 10 m² could potentially enter the seed bank. Heavy post-dispersal insect predation resulted in a decrease in viability of nearly 30% in exposed *P. haydenii* seeds after just 12 hours. *In situ* seed bank sampling showed that only 1 seed/ 10 m² actually persisted in the shallow soil seed bank. The presence or absence of a seed in the seed bank was not correlated with either adult plant density or sand dune movement. *Penstemon haydenii* does not appear to form an ecologically significant shallow seed bank and most likely relies on current year seed production and adult plant adaptations to survive in active sand dune habitats. It may form a deeply buried long-term seed bank through secondary dormancy induction during burial, but the relatively shallow sampling depth used in this study did not permit evaluation of this possibility.

INTRODUCTION

Land-use changes in the past century have resulted in sand dune stabilization and the loss or endangerment of many rare and endemic sand dune species (Arens and Geelen, 2006; Grootjans et al. 2002; Levin and Ben-Dor, 2004). Conserving sand dune plant diversity has therefore become a cause for concern for many land managers working in active sand dune systems. In these systems seed populations in the soil alter the structure and dynamics of the plant community (Leck et al., 1989). The formation of a persistent soil seed bank, i.e. where in seeds survive in the soil for more than a single year, may be important in habitats that experience frequent, unpredictable disturbances (Fenner and Thompson, 2005; Kottas, 2008). In these habitats delayed germination can act as a “bet hedging” strategy in that it can lower the likelihood that all seedlings will die before reproducing in the event of a catastrophic disturbance (Clauss and Venable, 2000). Active sand dunes are habitats in which burial by sand is a frequent disturbance. Burial imposes an abiotic stress on the plants living therein (Maun, 1998). One strategy plants can adopt to combat this stress is to form a persistent seed bank as a buffer against population extinction. Understanding the seed bank ecology of species living in active sand dunes systems can play a critical role in species conservation and the management of these systems (Bai et al., 2004; Junling and Zhimin, 2008; Qiaoling et al., 2005).

In order to better understand the ecology of the soil seed bank, the potential input of seeds into and the magnitude of seed loss prior to entrance into the persistent soil seed bank needs to be characterized. Actual viable seed output per plant is affected by both intrinsic limitations, such as genetically programmed ovule abortion (Weins et al., 1989) and extrinsic limitations, such as resource availability, pollinator availability, and predation (Ackerman, 1989). Seed production, a measure of how many viable seeds are produced per adult plant,

provides an estimate of the number of seeds that potentially enter the soil seed bank. Rodent and insect predation can remove large quantities of seeds post-dispersal and can significantly reduce seed inputs into persistent seed banks (Abramsky, 1983; Auld and Denham 1999; Inouye et al., 1980). North American sand dune habitats typically host from one to five or more species of seed-eating rodents (Brown, 1973). Predation studies can provide an estimate of how much seed is being removed or destroyed post-dispersal by both rodent and insect predators.

Penstemon haydenii (blowout Penstemon) is an endangered sand dune species endemic to the sand hills of west-central Nebraska and the Ferris dunes system of Carbon County, Wyoming (Heidel, 2012). It is an early seral, perennial species associated with sparsely vegetated, shifting dunes with active wind erosion (Heidel, 2012). As a species endemic to active sand dunes and blowouts, *P. haydenii* populations are subjected to frequent burial by sand. It is possible that this species has adopted a persistent soil seed bank to prevent local extinction under the occasional catastrophic burial event. Kottas (2008) performed field work on *P. haydenii* populations in the Nebraska sand hills and suggested that *P. haydenii* had the potential to form a persistent seed bank based on the fact that ungerminated seeds retained viability in the soil for multiple years. However, the results from Kottas' work also showed that this possibility was unlikely, since most viable seeds germinated within the first year following dispersal (Kottas, 2008). Following 4 weeks of moist chilling, *P. haydenii* seeds germinated to nearly 100% in the laboratory (Tilini, K., Brigham Young University, unpublished data). Seeds in the field are likely to experience similar conditions under winter snowpack and few seeds would be expected to remain ungerminated.

We performed a series of studies along an active sand dune transect to characterize the seed bank ecology of *P. haydenii*. The goals of our study were to (1) estimate the annual seed

rain, (2) assess the magnitude of seed loss due to predation, and (3) measure seeds in the shallow seed bank and determine where those seeds were located in relation to *P. haydenii* adult plant density and sand dune movement. We hypothesized that a large proportion of the viable *P. haydenii* seeds produced by each plant would be taken by rodent and insect predators prior to entrance into the soil seed bank. We also hypothesized that the active seed bank would be relatively small and finally, that any *Penstemon haydenii* seeds in the persistent seed bank would more likely be found in areas with high adult plant density and moderate rather than extreme sand dune movement.

MATERIALS AND METHODS

Study Site:

Our study was performed on the Bear Mountain population of *P. haydenii* located in the Ferris Dunes system of Carbon Co., Wyoming (Longitude: -107.07086, Latitude: 42.24348, elevation: 2100 m). The Ferris Dunes consist of > 300 km² of predominantly stabilized parabolic sand dunes downwind of the Great Basin Divide (Stokes and Gaylord, 1993). *P. haydenii* occupies active sand dune slopes ranging in elevation from 1786-2270 m. The Bear Mountain population occurs on the steep slip-faces of dunes with a slope > 60% (Heidel, 2012) and soils consisting of psamments derived from wind-blown Quaternary alluvium (Munn and Arnesen, 1998).

Climate data were obtained from a nearby station at the Rawlins Airport, which corresponds closely with the Ferris Dunes' elevation and setting. Mean annual precipitation is 23.4 cm, with a peak in April and May. Mean annual temperature is 6 °C. Mean minimum and maximum January temperatures are -10.7 °C and -0.6 °C respectively, and mean minimum and maximum July temperatures are 10.8 °C and 28.7 °C respectively (Heidel, 2012).

P. haydenii inhabits sparsely vegetated slopes with less than 5% plant cover (Fertig, 2001). Predominant species associated with *P. haydenii* occurrence are blowout grass (*Redfieldia flexuosa*), indian ricegrass (*Achnatherum hymenoides*), thickspike wheatgrass (*Elymus lanceolatus*), and lemon scurf-pea (*Psoralidium lanceolatum*, Heidel, 2012).

Seed bank estimates:

To characterize the seed bank of the Bear Mountain *P. haydenii* population we set up a transect line in May of 2012 that ran along the dune face, across the top and back side of the Bear Mountain Dune. 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. Planting stakes served not only as markers for each sampling point but were also used to measure sand movement along the dune. 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 stake heights allowed us to measure sand movement at each point along the transect line and therefore relate this movement to plant density and 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 not included in this 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 sifted on site using round metal sieves with a mesh size of 0.28 cm (7/64 in). Seeds and debris left on the sieve were collected in a labeled bag and transported to our laboratory for further processing. *P. haydenii* seeds found at each sampling point were counted and recorded. A total surface area of 20 m² of soil was processed to a depth of 10 cm to estimate the persistent soil seed bank.

Data collected on the presence or absence of a *P. haydenii* seed in each sample was analyzed in relation to with both sand dune movement and adult *P. haydenii* plant density along the transect using Generalized Linear Models (GLM). A Linear Model (LM) was used to compare *P. haydenii* adult plant density with sand dune movement. Comparisons with a probability level of $P < 0.05$ were considered significant. Analyses were performed using the software R 2.15.2 (R Development Core Team, 2012).

Seed rain and reproductive success:

To estimate seed rain for the Bear Mountain *P. haydenii* population we took a series of four measurements that were then used to determine the number of viable seeds produced per unit area in a single season. The first measurement determined the number of plants per unit area. In June 2012, a tape was used to extend 4.6 m on either side of the 399 m transect for a total area of approximately 3,650 m². The total number of adult plants within this area was counted as we moved from sampling point to sampling point. The second measurement determined the ratio of reproductive to non-reproductive plants. This was obtained by scoring the number of reproductive plants out of subsample of 500 plants counted in November 2012. The third measurement was to determine the number of stalks per reproductive plant. To do this we counted the total number of stalks on the three plants nearest to each sampling point within 4.6 m in July 2012. We then randomly collected one stalk from each of the three plants, placed it in a labeled brown paper sack, and took it to the laboratory to determine the number of seeds per stalk. Once all measurements were obtained, we combined them to estimate the seed rain of the Bear Mountain *P. haydenii* population.

In the laboratory, factors affecting the reproductive output of individual *P. haydenii* plants were also measured and averages were calculated. The length of each stalk (cm) was measured along with the following for each collected stalk: number of filled capsules and seeds, number of aborted capsules and seeds, and number of insect-damaged capsules and seeds. These measurements allowed us to estimate both ovary (capsule) and ovule (seed) success.

Post-dispersal predation:

The incidence of post-dispersal insect and rodent predation for this population was determined in field exclusion experiments. In these trials, ten replications of 25 seeds each were subjected to one of four exclusion treatments: (1) exclusion of insect and rodent predators, (2) exclusion of rodent predators only, (3) exclusion of insect predators only, and (4) no exclusion.

In July of 2012 ten blocks containing one replication of each of the above treatments were established every fourth sampling points on the transect line, along the dune face only. We assumed this is where *P. haydenii* seed predators would most likely be found due to a greater abundance of adult plants in this area. For each treatment, seeds were placed on the surface of 15 X 100 mm plastic petri dishes filled with sand. The bottoms of the plastic petri dishes were glued inverted to the top of the dish. For insect exclusion, Tanglefoot™, an insoluble sticky substance, was smeared around the outside rim of the Petri dish bottoms and kept above the soil surface. To provide access to insects, petri dishes were slightly buried in the sand to make the surface even with the soil. Wire mesh cages were assembled and placed over petri dishes to exclude rodent predators. The cages were secured into the sand using large, metal garden staples.

A series of four trials were conducted over a three day period. The first trial was started on Tuesday evening and retrieved Wednesday morning. The second trial was started Wednesday

morning and retrieved Wednesday evening. The third trial was started Wednesday evening and retrieved Thursday morning. The last trial was started Thursday morning and retrieved Thursday evening. Insect predators are more likely to be active during the day, whereas rodent predators tend to be nocturnal. In order to account for possible diurnal and nocturnal differences in predation we included two night trials and two day trials. Seeds were exposed to predation for approximately 12 hours. Following exposure, seeds were collected in labeled coin envelopes and taken back to the laboratory for viability testing using a cut test (Association of Official Seed Analysts, 1988) and tetrazolium staining (Grabe, 1970).

The *P. haydenii* predation data were analyzed using a General Linear Model in SAS v. 8.1 (PROC GLM; SAS Institute, 2000). The effects of treatment, block, and time of day on the proportion of damaged *P. haydenii* seeds were examined. Differences with a probability level of $P < 0.05$ were considered statistically significant.

RESULTS

Seed bank estimates:

A total of 21 *P. haydenii* seeds were recovered from the approximately 20 m² surface area of sand sampled to a depth of 10 cm in the seed bank analysis. Only 2 of the seeds were viable. This gave us an estimate of approximately 1 viable seed for every 10 m² surface area for the shallow persistent seed bank for this population of *P. haydenii*.

Average adult plant density surrounding samples with a seed present in the sample was slightly greater (0.14 plants/m²) than for samples without a seed present (0.13 plants/m²). However, this relationship was not significant ($P = 0.781$) and the presence or absence of a seed within any given sample was not correlated with the density of adult plants surrounding the sample (Fig. 1).

Average net sand movement for samples with a seed present was also slightly greater (2.8 cm.) than samples without a seed present (0.5 cm). However, this relationship was also not statistically significant ($P = 0.199$) and the presence or absence of a seed within any given sample was not directly correlated with the average net sand movement at the sample site (Fig. 2). There was no correlation between average adult plant density and net sand dune movement ($P = 0.914$; Adjusted R-squared = -0.019). However, there were no adult plants present where the sand dune had nearly stabilized and almost no net sand movement was measured (Compare Figs. 1 and 2).

Seed rain and reproductive success:

We counted a total number of 459 adult *P. haydenii* plants within the 3,650 m² belt run along our established transect line. This means there was approximately 1 plant every 10 m² in the area from which our samples were retrieved. A little over half of these adult plants were reproductive and produced on average 2.6 stalks and 70 seeds per stalk (Table 1). Using these measurements we estimated the seed rain for the Bear Mountain *P. haydenii* population in 2012 to be approximately 14 seeds/m². Average stalk height was 6.3 cm (Standard Deviation: 2.95 and Coefficient of Variation: 0.47). Ovary (capsule) success for this population appeared quite low; only a little over a third of the capsules actually yielded seeds. Over half were aborted and a small percentage was damaged by insects to the extent that individual seeds could not be distinguished and counted (Fig. 3). The ovule (seed) success for this population was higher; over half the seeds produced in each capsule were filled (Fig. 3). A third of the seeds were aborted and a small percentage was damaged by insects (Fig. 3).

Post-dispersal predation:

In our predation study, the treatment intended to exclude insect access to seeds (Tanglefoot™) failed. As the dishes were placed on the sand surface wind blew sand onto the sides of the dishes, coating the Tanglefoot™ in a thick layer of sand and actually providing insects with a way to access the seeds within each dish. The wire-mesh cages meant to exclude rodent access did not fail; however, no rodent predation was detected in any of the treatments and no seeds were taken from these dishes. Since no seeds were taken from any of the dishes we assumed predation by birds was irrelevant. There were no significant differences in seed predation among any of the treatments ($p=0.1582$). However, there was evidence of insect

predation in all treatments. Viability dropped from an initial 93% to an average of 66% following approximately 12 hours of exposure on the dune. This represents a reduction in viability of 27%. Cut tests in the laboratory showed seed damage characteristic of insect predation, i.e. shriveled sections of the embryo and chewing damage to the endosperm and embryo.

DISCUSSION

Our results indicate that *P. haydenii* does not form an ecologically significant, persistent seed bank. A single seed buried every 10m² would likely not be sufficient to restore this population in the event of a large-scale burial leading to a loss of the adult population. We believe that heavy insect predation and high spring germination rates following winter chill most likely account for the lack of significant seed quantities in the soil. Four weeks of moist chilling yielded almost complete germination of *P. haydenii* seeds in the laboratory (unpublished data). Moist chilling closely imitates conditions under winter snowpack and is required to break primary dormancy in many *Penstemon* species (Meyer, et al., 1995). Field work performed by Kottas (2008) on the longevity of *P. haydenii* seeds in the soil revealed that a majority of viable seeds germinated within the first year following planting. *P. haydenii* seeds appear to possess predictive dormancy mechanisms that ensure germination under optimal spring conditions following winter chill. However, they do not appear to possess carry-over mechanisms that would ensure that a portion of the seeds remain in primary dormancy and enter the persistent seed bank. Seed carry-over for perennial plants is not as crucial to survival as for annual plants, because surviving adult vegetation can buffer the population against less than optimal seed years (Venable and Lawlor, 1980). Adult plants of coastal dune species have been shown to possess adaptations that allow them to emerge from burial (Maun, 1998). Some of these adaptations include elongation of buried internodes, development of horizontal rhizomes containing carbohydrate reserves for emergence from burial, and elongation of petioles to expose photosynthetic leaf tissues (Maun, 1998). Short-lived perennial species tend to rely more heavily on current year seed for population regeneration than on persistent seeds in the seed bank (Louda and Potvin, 1995). As a short-lived perennial (Stubbendieck et al., 1997) *P. haydenii* is more

likely to depend on current year seed and spring germination for population regeneration.

However, there is the possibility that a persistent seed bank for this species exists at burial depths greater than the relatively shallow depths we measured in this study. If *P. haydenii* seeds become deeply buried before receiving the chilling cue necessary for breaking primary dormancy, they could fail to germinate and become a part of the persistent seed bank.

Heavy predation of post-dispersal seeds is also likely to reduce the number of viable seeds entering the persistent seed bank. The results from our predation study revealed that post-dispersal insect predation accounted for an average reduction in viability of *P. haydenii* seeds of nearly one third in a single day. The control in this experiment failed and all treatments were subjected to insect predation. However, we were still able to get an estimate of viability reduction due to insect predation by comparing initial viability of the seeds with viability post-exposure. Seeds were only in the experiment for approximately 12 hours, making it highly unlikely that factors other than insect predation could account for such a high loss in viability. Also, there was visual evidence of insect predation on the seeds during viability testing. This estimate of insect seed predation is, however, likely to be higher than what we would expect in the natural system for two main reasons: 1) seeds do not typically remain in concentrated piles post-dispersal and 2) seeds are most likely buried shortly following dispersal. However, given a few months in the field, if the seeds remain at the soil surface, insects could have quite a large impact on seed survival. In sand dune systems wind and sand dune action combine to have a significant effect on the horizontal distribution of dispersed seeds (Qiaoling et al., 2005). Seeds are distributed across the landscape away from the parent plant and each other which decreases seed density in any given area. As food density decreases, an animal spends more time searching for food and therefore, fewer food items are actually consumed (Norberg, 1977). Increased

foraging time for insects due to searching for seeds spread out across the landscape would have resulted in a lower estimate of the effect of insect predation on *P. haydenii* seeds. In active sand dune systems, wind and sand action work in concert to not only distribute seeds horizontally but also vertically in the soil profile (Junling and Zhimin, 2008). Once seeds are buried (> 1 cm) they are no longer susceptible to insect predation (Abramsky, 1983). This would also result in a lower estimate for the extent of insect predation on *P. haydenii* seeds than resulted in our study.

Our predation field study provided no evidence of rodent predation for this population of *P. haydenii*. However, this does not mean there is no rodent predation. Rodent predation on *P. haydenii* seeds was recorded by Kottas (2008) in field studies in Nebraska. Major rodent predators encountered in Kottas' study were Ord's kangaroo rats (*Dipodomys ordii*) and deer mice (*Peromyscus maniculatus*) (2008). Mice signs were reported in *P. haydenii* habitat in Wyoming (Heidel, 2012) and it is probable that Wyoming populations of *P. haydenii* also experience rodent predation. The absence of rodent predation in our field study was most likely due to the timing of our sampling. Our predation study was performed before natural dispersal occurred, meaning that the rodents would likely not yet be foraging for seeds on the dune.

The location of *P. haydenii* seeds in the seed bank was not correlated with sand dune movement or plant density. This is most likely due to seed dispersal away from the host plant by wind and sand movement. In active sand dune systems, the action of wind and resulting sand movement redistribute seeds in the soil and act as the most important abiotic factors controlling seed distribution (Qiaoling et al., 2005). Therefore, there is often little similarity between species composition of seeds in the seed bank and species composition of aboveground vegetation in these systems (Qiaoling, 2005). Small sample sizes, or low absolute numbers seeds in the seed

bank, could also account for the lack of correlation between seed bank samples and plant density or sand movement.

Sand dune movement and adult *P. haydenii* plant density were not linearly correlated. However, no *P. haydenii* plants were found in areas where there was no sand dune movement. This occurred across the top of the dune toward the end of the transect line. As a colonizing early seral species, *P. haydenii* is well adapted to and establishes on active sand dunes prior to stabilization; once the dunes become stabilized and other species colonize, it can no longer compete for resources and disappears from these areas (Weedon et al., 1982). In addition, there were few or no *P. haydenii* plants below the ridge crest of the dune, where sand deflation was most marked.

We estimated the potential seed rain of *P. haydenii* to be approximately 14 seeds/m². This means that for every 10 m² up to 140 seeds could enter the soil seed bank. The potential reproductive capabilities of this population of *P. haydenii* are much lower than those estimated for populations in Nebraska. Kottas (2008) estimated an average of 531 viable seeds per plant from two Nebraska sites, Hooker County and Cherry County. The Nebraska estimate is much higher than our estimate of only 182 viable seeds per plant for the Bear Mountain, Wyoming population. Average stalk height estimates were also much lower for our Wyoming population, only about one third that of the Nebraska populations. This Wyoming population of *P. haydenii* may possess lower reproductive potential than the Nebraska sites due to adaptations that enable it to live in a more extreme environment. The elevation for our Wyoming site is over 1,000 m higher than the Nebraska sites and yet receives almost 200 mm less precipitation on average each year. These differences in elevation and climatic conditions could have led the Bear Mountain

P. haydenii population to become genetically dwarfed in comparison to Nebraska populations. Plant populations may respond to stressful environments through the genetic adaptation of ecotypes (Bennington and McGraw, 1995). Decreased plant size in response to increased elevation is a well-known phenomenon (Hautier, 2009) and studies have shown empirically that reproductive effort often varies with size as reported by Samson and Werk (1986). It is possible that Wyoming populations of *P. haydenii* have developed ecotypes in response to living at much higher elevations than their Nebraska counterparts. Reciprocal transplant and common garden studies would be needed to determine if there is a genetic basis for the differences in size and reproductive potential.

Over half of the flowers produced by adult *P. haydenii* plants were aborted and failed to set seed. Many species of flowering plants regularly produce more flowers than actually become mature fruits and set seed (Stephenson, 1981). Possible reasons for this phenomenon were reported by Santon et al. (1981) and include both evolutionary (increased fruit production under favorable conditions and increased pollination with more attractive displays) and mechanistic (pollen and resource limitations to seed set) explanations. Studies on the pollination biology of *P. haydenii* found no indication of resource or pollination limitations to reproduction for Nebraska populations (Tepedino et al., 2006) and mechanistic explanations for low capsule success seem unlikely. *P. haydenii* is known to be one of the few self-incompatible *Penstemon* species and requires pollination for successful reproduction (Tepedino et al., 2006). As a self-incompatible species, attracting pollinators is of high priority and it is possible that *P. haydenii* produces greater numbers of flowers than can produce seed in as an adaptation to more effectively attract pollinators by visual stimulation or nectar rewards. The activity of pollinator species has been linked to nectar production in flowering plants (Real and Rathcke, 1991;

Varassin et al., 2001) and larger inflorescences have been shown to be visited by more pollinators than smaller ones (Wilson and Bertin, 1979). *P. haydenii* is also one of the few fragrant species of *Penstemon* and may use volatile compounds in its flowers to attract pollinators. Another explanation for *P. haydenii* low capsule success could be a bet hedging strategy in which the plant produces large numbers of potential capsules in the event of a good resource year. Flower and fruit abortion enable a plant to coordinate seed production with resource availability under variable conditions from year to year (Stephenson, 1981). As a sand dune endemic, *P. haydenii* could encounter resource availability that is likely to be highly variable and could be implementing this strategy to coordinate seed production with changing resource availability.

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TABLE AND FIGURE CAPTIONS

Table 1: Measurements taken to estimate seed rain for the Bear Mountain *P. haydenii* population. Seed rain estimate was calculated by multiplying together the four primary measurements.

Figure 1: Adult plant density/m² surrounding each sample point along the established Bear Mountain transect line. The stacked stars above each bar indicate the number of seeds found in the persistent seed bank at each sampling point. “P” in the x-axis refers to an individual post or sampling point along the transect.

Figure 2: Extent of sand dune movement measured in cm. at each sampling point between visits to the Bear Mountain site. The differences in sand movement for each line on the graph are in relation to the previous read. Sampling points at which the line is above 0 experienced burial during the measured period and sampling points at which the line falls below 0 experienced erosion. “P” in the x-axis refers to an individual post or sampling point along the transect.

Figure 3: Factors affecting the reproductive output of *P. haydenii*. The left bar represents ovary (capsule) success and the right bar represents ovule (seed) success.

TABLES AND FIGURES

Table 1

Measurement	Value	Standard Deviation	Coefficient of Variation
Plants/m ²	0.126 plants	11.86	1.47
Reproductive Plants/ Total Plants	0.613 plants	2.84	4.63
Stalks/Reproductive Plant	2.61 stalks	2.96	1.14
Seeds/Stalk	70.01 seeds	71.01	1.01
Seed Rain Estimate	14.11 seeds/m ²	NA	NA

Figure 1

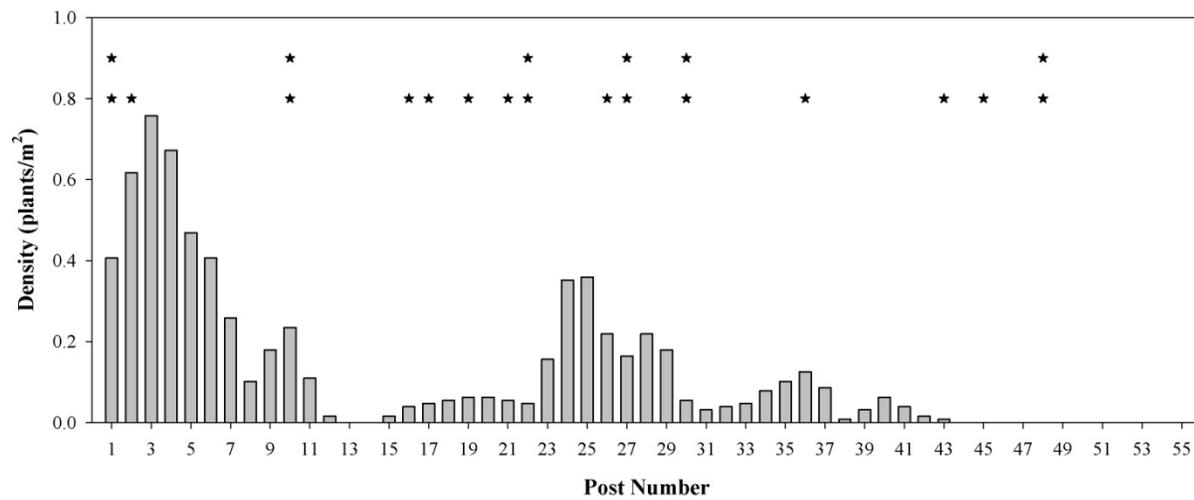


Figure 2

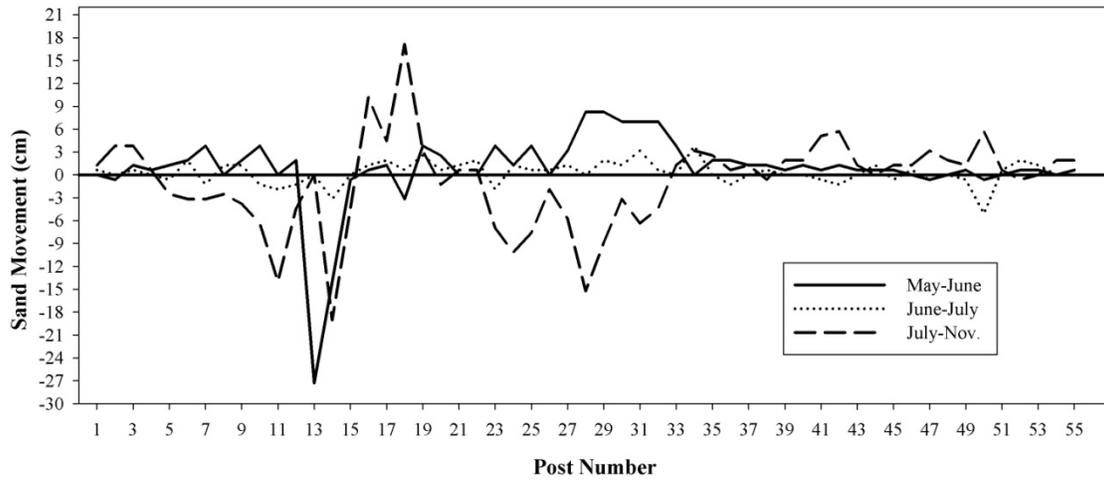
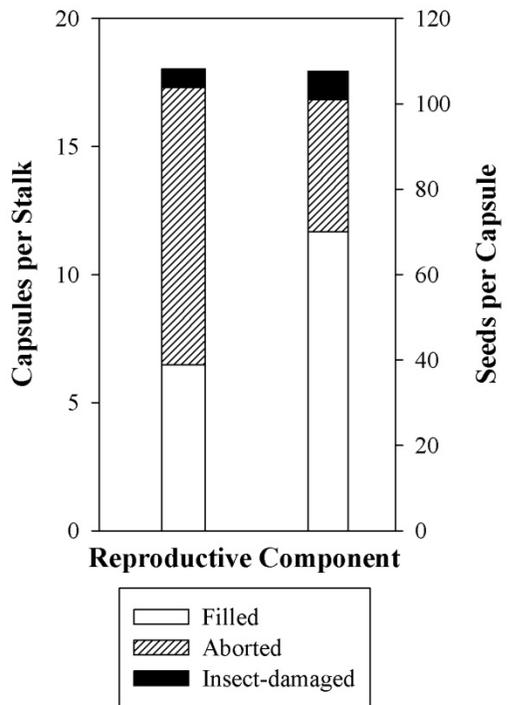


Figure 3



CHAPTER 3

The effects of burial by sand on *Penstemon haydenii* (Scrophulariaceae) seed dormancy and seedling emergence

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ABSTRACT- ARTICLE 3

Active sand dunes are habitats in which burial by sand plays an important role in regulating seed dormancy and seedling emergence. This study was conducted to better understand how sand burial depth affects seed dormancy and seedling emergence of a rare sand dune endemic, *Penstemon haydenii*. Wild-harvested seeds were planted in pots at 1, 2, 4, 6, 8, and 10 cm deep in sand, and then incubated at 10-20 °C in a growth chamber. Seed germination, seedling emergence, and mortality were measured and the response of ungerminated seeds to post-burial incubation was determined. The proportion of *P. haydenii* seedling emergence decreased with increasing burial depth. Failure to emerge was mainly due to failure to germinate. A majority of deeply buried (8 and 10 cm) seeds (> 60%) experienced enforced dormancy in burial but were germinable in post-burial incubation. A smaller fraction (< 38%) was induced into secondary dormancy. Shallowly buried seeds (1 and 2 cm) either germinated and emerged from burial (37-77%) or were induced into secondary dormancy (>85%). Few ungerminated seeds (< 6%) retrieved from shallow burial germinated in incubation. Seeds from intermediate depths (4 and 6cm) showed an intermediate response in post-burial incubation; about 30% remained germinable and about 70% were induced into secondary dormancy. Seed burial greater than 4cm dramatically suppresses *P. haydenii* germination and the seeds of this species appear to possess a depth sensing mechanism which inhibits germination under unfavorable burial conditions.

INTRODUCTION

Active sands dunes are habitats in which wind erosion and sand movement play an important role in distributing seeds and plants both horizontally and vertically across the landscape (Qiaoling et al., 2005, Junling and Zhimin, 2008). In these systems, burial by sand is a frequently recurring phenomenon and acts as strong selective force for plant adaptation (Maun, 1998). Seed dormancy protects more vulnerable plant life stages from unfavorable environmental conditions (Vegis, 1964). One such condition is represented by excessive burial that imposes abiotic stresses such as changes in soil compaction, pH, and oxygen availability on seedling survival (Maun, 1998). One mechanism seeds can employ to avoid the stresses associated with germination under excessive burial is to remain dormant until conditions become more favorable for germination and seedling establishment, i.e. the seeds regain proximity to the surface. Studies performed on a number of sand dune species showed that increasing burial depth correspondingly increased enforced dormancy of seeds and suppressed germination (Pemadasa and Lovell, 1975; Jianhua and Maun, 1990; Hua and Maun, 1999; Jun et al., 2002). In this paper we define “enforced dormancy” as a condition in which germinable seeds are prevented environmentally from germinating but will resume normal germination when favorable conditions are restored. Secondary dormancy is another form of dormancy in which initially nondormant seeds or those that have been rendered nondormant are induced into dormancy under certain conditions (Baskin and Baskin, 1985). Seeds rendered secondarily dormant will not germinate even when placed under favorable conditions until secondary dormancy has been broken. Excessive burial has also been shown to induce secondary dormancy in a number of weed species (Benvenuti, 2003).

Burial by sand can strongly affect seedling emergence and survival. Once germinated, seedlings rely solely on seed energy reserves to reach the soil surface and the size of these reserves dictates from what depth seedlings can emerge (Maun and Lapierre, 1986). Numerous studies on sand dune and weed species show that as burial depth increases, seedling emergence decreases (Maun and Lapierre, 1986; Jianhua and Maun, 1990; Maun, 1998; Hua and Maun, 1999; Benvenuti et al., 2001; Jun et al., 2002; Benvenuti, 2003).

Blowout penstemon (*P. haydenii*) is a rare perennial herb endemic to the sand hills of west-central Nebraska and the Ferris sand dunes of Carbon County, Wyoming (Heidel, 2012). It is an early seral species directly associated with sparsely vegetated, shifting sand dunes with active wind erosion (Heidel, 2012). *Penstemon haydenii* was listed as Endangered by the U.S. Fish and Wildlife Service in 1987 and is protected under state law in Nebraska (Heidel, 2012). As a sand dune endemic, it is likely that burial by sand plays an important role in limiting the seed germination and seedling emergence of this species.

The goal of this study was to examine the effects of burial by sand on seeds and seedlings of *P. haydenii*. The specific objectives of our study were to determine the effects of burial depth on (1) seed germination, dormancy and mortality, (2) seedling emergence and mortality, (3) germination, dormancy, and mortality of retrieved seeds in post-burial incubation, and (4) the success of re-chilling on breaking secondary dormancy. We hypothesized that as burial depth increased (1) seed germination would decrease and enforced dormancy would increase, (2), seedling emergence would decrease and mortality would increase, and (3) induced secondary dormancy of seeds in post-burial incubation would increase.

MATERIALS AND METHODS

Seed Source:

Seeds of wild *P. haydenii* plants were collected in July of 2012 from the Bear Mountain sand dune, a member of the Ferris dunes system in Carbon Co., Wyoming (421425.25 N and 1070426.682 W). To collect seeds, a transect line was run through the *P. haydenii* population and sampling points were established every 7m. At each sampling point one randomly chosen seed stalk from each of the three nearest plants within 3m was removed. Stalks were clipped at the base, placed in a brown paper sack, and transported to the laboratory. Seed capsules were removed from the stalks and seeds were cleaned using screening and blowing. 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 (Grabe, 1970).

Experimental Procedure:

Wild-harvested *P. haydenii* seeds exhibit primary dormancy. To break primary dormancy, seeds intended for this study were placed on water-saturated germination blotters in 15 X 100 mm plastic Petri dishes. Dishes were then placed in a dark growth chamber at 2-4 °C for four weeks, which preliminary experiments showed will effectively break primary dormancy and stimulate seed germination to between 98 and 100% (Tilini, K. Brigham Young Univeristy, unpublished manuscript).

Following moist chilling, seeds were removed from Petri dishes and subjected to one of six burial depth treatments: 1, 2, 4, 6, 8, or 10 cm. Seeds were placed in plastic planting pots

(13 x 12 cm) and buried with sand to the assigned treatment depth. Sand used in this experiment was obtained directly from the Bear Mountain site where seed collection occurred. Pots were then placed in trays filled with wet sand and buried approximately half-way in 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 put into a 10-20 °C growth chamber (12 hour photoperiod). Pots were watered every other day to saturation and seedling emergence was recorded daily for six weeks at which time the experiment was ended and seeds and seedlings were exhumed. At the end of the experiment, the sand in each pot was washed through a fine sieve to retrieve both the ungerminated seeds and the unemerged seedlings. Seeds were categorized as ungerminated or dead and seedlings as emerged or unemerged (germinated but failed to emerge).

Ungerminated seeds retrieved from burial were then placed in 15 X 100 mm plastic Petri dishes on saturated germination blotters and put back into 10-20 °C incubation for four weeks to test for burial-induced secondary dormancy. Dishes were read twice a week and scored for germination. Following 4 weeks in incubation seeds were categorized as germinated, dormant, or dead. Seeds that experienced suppressed germination in burial but then germinated in post-burial incubation were considered in a state of “enforced dormancy.” Seeds remaining dormant in post-burial incubation were considered in a state of “secondary dormancy” and were then subjected to re-chilling (2-4 °C) in an effort to break this dormancy. Dead seeds were those which were visibly infected with either bacteria or fungal colonies and were soft to touch. Firm seeds were considered alive and dormant and were included in re-chilling. Prior to initiation of re-chilling, five seeds from each treatment replication containing ten or more dormant seeds were tested using tetrazolium staining (Grabe, 1970) to verify viability. Following four weeks of re-chilling, seeds were placed back into 10-20 °C incubation and read twice a week for germination.

Statistical analysis:

Data collected on seed germination and mortality and seedling emergence of *P. haydenii* seeds in burial was converted to proportions of total seeds planted. Data collected on post-burial incubation seed germination, mortality and dormancy were converted to proportions of the number of ungerminated seeds recovered from burial. These same variables were also analyzed as the proportions of total number of seeds planted in burial to ensure patterns were not simply artifacts of high germination in burial. Data collected from re-chilled seeds were converted to proportions of the number of seeds remaining dormant in post-burial incubation. Proportional data from burial, incubation, and re-chilling were arcsine square root transformed prior to analysis. All data were analyzed in a randomized block design in SAS v. 8.1 (PROC MIXED: SAS Institute, 2000). Models were run to determine the effects of varying burial depths on the previously mentioned variables. Separate models were run for seed response to burial, post-burial incubation, and re-chilling. LSMEANS separations were run on differences among burial depths.

RESULTS

***P. haydenii* seed response to burial:**

As burial depth increased (up to 4 cm), seed germination decreased ($p < 0.0001$, $F = 12.65$, $DF = 18$). At all burial depths greater than 4cm nearly all seeds ($> 90\%$) experienced suppressed germination (Figure 1B). Burial depth up to 4 cm also significantly decreased the proportion of emerged seedlings ($p < 0.0001$, $F = 13.12$, $DF = 18$) (Figure 1A). Failure of seedlings to emerge was mostly due to failure of seeds to germinate since very few seeds germinated at depths greater than 4 cm. However, for the few seeds that did germinate and fail to emerge, 2, 4, and 6 cm depths yielded higher proportions of unemerged seedlings than 8 and 10 cm depths ($p = 0.0427$, $F = 2.91$, $DF = 18$) (Figure 1C). This is likely an artifact of the fact that so few seeds germinated from 8 and 10 cm. Mortality was so low at all burial depths that no treatment effect could be discerned ($P = 0.7590$, $F = 0.52$, $DF = 18$) (Figure 1D).

***P. haydenii* response to post-burial incubation:**

The proportion of retrieved seeds held under enforced dormancy during burial but germinating in post-burial incubation increased with increasing burial depth ($p < 0.0001$, $F = 11.45$, $DF = 17$). Deep burial (8 and 10 cm) had the highest proportion of germinated seeds in post-burial incubation, followed by 4 and 6 cm, while 1 and 2 cm had the fewest (Figure 2A). Conversely, the proportion of seeds rendered secondarily dormant and failing to germinate in post-burial incubation decreased with increased burial depth ($p = 0.0022$, $F = 6.01$, $DF = 17$). The two deepest burial treatments had the smallest proportion of seeds that remained dormant in post-burial incubation followed by the two intermediate depths (4 and 6 cm), while the two

shallowest depths had the most (Figure 2B). Mortality was not significantly affected by burial depth ($p = 0.9430$, $F = 0.23$, $DF = 17$) and very few seeds died in post-burial incubation (Figure 2C). Even when analyzed over the total number of seeds planted in burial, there was a treatment effect on the proportion of seeds rendered secondarily dormant ($p = 0.0428$, $F = 2.91$, $DF = 18$).

***P. haydenii* response to re-chilling:**

Re-chilling *P. haydenii* seeds that became secondarily dormant as a result of burial slightly stimulated germination. Seed burial depth affected the small proportion of seeds that responded to re-chilling ($p = 0.022$). Six cm burial depth had the highest proportion of seeds germinating, almost 11% while all other burial depths yielded 3% or less germination.

DISCUSSION

As seed burial depth increased, the proportion of *P. haydenii* seedlings emerging decreased. A burial depth of only 2 cm halved seedling emergence and depths greater than 4 cm almost completely inhibited emergence. The depth from which seedlings can potentially emerge has been associated with both seed size (Maun and Lapierre, 1986; Hua and Maun, 1999) and energy reserves (Maun, 1998). Larger seeds typically have greater energy reserves and are able to emerge from greater depths of burial. Despite the relatively large size of *P. haydenii* seeds, it appears that relatively shallow burial is enough to inhibit germination. *Penstemon haydenii* inhabits relatively harsh environments and seedlings must be able to emerge and then survive under the extreme conditions associated with high elevation, active sand dunes. These conditions include frequent wind erosion, sand abrasion, high surface temperatures, drought, and sun exposure. Excessive burial of seeds has been shown to have negative effects on seedling establishment (Jianhua and Maun, 1990). Larger seeds emerging from shallower depths would be able to produce more extensive root systems to absorb nutrients and water (Hua and Maun, 1999). Failure of *P. haydenii* seedlings to emerge was due mostly to failure to germinate. Very few germinated seeds actually failed to emerge suggesting that attempted emergence from deep burial has a negative effect on plant survival or fitness and is, therefore, tightly regulated. It appears that *P. haydenii*, like many other species (Maun and Lapierre, 1986; Jianhua, 1990; Maun, 1998; Hua and Maun, 1999; Jun et al., 2002), possesses some type of depth sensing mechanism or response which inhibits germination under the unfavorable conditions associated with excessive burial.

The mediation of seed germination inhibition through burial depth has been linked to a number of potential factors. One such proposed factor is sensitivity to temperature fluctuations in

the soil. Studies performed on *Sorghum halapense* seed germination showed that sensitivity of seeds to temperature fluctuations in the soil was a major component of its depth sensing mechanism (Ghersa et al., 1992). Temperature fluctuations in the soil are dependent on depth (Thompson, 1977; Ghersa et al., 1992) and the seeds of many species have been shown to positively respond to fluctuating incubation temperatures (Thompson and Grime, 1983). *Penstemon haydenii* has been shown to exhibit a preference for cool diurnally fluctuating incubation temperatures for optimum germination (Tilini, K., Brigham Young University, unpublished manuscript). As temperature fluctuations decrease with increasing depth, it is plausible that *P. haydenii* sensitivity to temperature acts as a mechanism for sensing burial depth and inhibiting germination.

Another proposed factor affecting depth-mediated germination inhibition is soil oxygen content and gas exchange capabilities. Studies performed on the effects of hypoxic conditions on seed germination attributed germination inhibition to increasing levels of hypoxia surrounding seeds (Benvenuti and Macchia, 1995). However, it was postulated that oxygen supply was of less importance and the main inhibitor of seed germination was due to poor gas exchange and therefore, inability to eliminate the products of anaerobic metabolism (Benvenuti and Macchia, 1995). These findings were supported in a related study that showed a close relationship between depth-mediated germination inhibition and poor gas exchange (Benvenuti, 2003). It is likely that *P. haydenii* germination inhibition at increasing burial depths is associated with reduced gas exchange capabilities of the soil in which the seed resides.

Penstemon haydenii seed response to post-burial incubation revealed some interesting patterns. A majority of ungerminated seeds planted at shallow burial were induced into secondary dormancy. Seeds failed to germinate even when restored to optimum conditions.

Ungerminated seeds retrieved from intermediate depths showed an intermediate response; about one third of the seeds were germinable and two thirds were induced into secondary dormancy. Lastly, seeds recovered from deep burial showed a high proportion of germination (over half), and a much smaller fraction entered secondary dormancy. A possible explanation for this pattern could be a race between two opposing processes: germination and secondary dormancy induction in the dark. At very shallow depths (1cm), where conditions are not limiting to germination, a majority of the seeds germinate rapidly. Only the slowest fraction of seeds is induced into secondary dormancy. At slightly deeper burial (2 cm) conditions are not as favorable to germination and germination is slowed. Under these conditions, over half the seeds are induced into secondary dormancy. At deep burial depths conditions are completely limiting to germination and possibly to a lesser extent, secondary dormancy induction. At these depths most seeds simply stay in a state of enforced dormancy and then germinate once restored to optimum germination conditions. The rest of the seeds are induced into secondary dormancy. At intermediate burial depths, conditions are limiting enough for enforced dormancy and very few germinate. However, the conditions are not limiting enough to prevent a large proportion of seeds from entering secondary dormancy. More research concerning secondary dormancy induction and its association with conditions limiting to germination at different burial depths would be needed to support this hypothesis and further explain this interesting pattern.

Re-chilling seeds in an effort to break primary dormancy was not effective for this population of *P. haydenii*. Very few secondarily dormant seeds germinated in re-chilling and the small proportion which did is ecologically insignificant. Placing seeds back into chill following a period of incubation has been shown to be ineffective in breaking primary and secondary dormancy in the seeds of many *Penstemon* species (Meyer, S., USDA Forest Service,

unpublished data). However, work on *Penstemon* propagation has shown that if the seed are first allowed to dry out for a period prior to re-chilling, a larger fraction will then germinate in incubation (Meyer, S., USDA Forest Service, personal communication). In the field buried seeds of *P. haydenii* probably never re-enter a state of moist chilling without drying out first. Seasonal fluctuations in temperature and moisture levels will subject seeds to a period of dry storage under the soil during summer before they re-enter moist chilling during the winter. It is possible that this response acts to prevent precocious germination during the summer and to ensure germination occurs during the optimum season, spring.

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TABLE AND FIGURE CAPTIONS

Figure 1: *P. haydenii* seed response to varying burial depths. Bar graphs represent the proportion of seedlings (\pm SE) that (A) emerged or (B) failed to emerge (unemerged) and the proportion of seeds (\pm SE) that (C) failed to germinate or (D) died in burial at varying depths.

Figure 2: Response to post-burial incubation of remaining ungerminated *P. haydenii* seeds following burial for 6 weeks. Bar graphs show the proportion (\pm SE) of retrieved *P. haydenii* seeds from different burial depths that (A) germinated (i.e. under enforced dormancy during burial), (B) were induced into secondary dormancy, and (C) died during post-burial incubation.

TABLES AND FIGURES

Figure 1

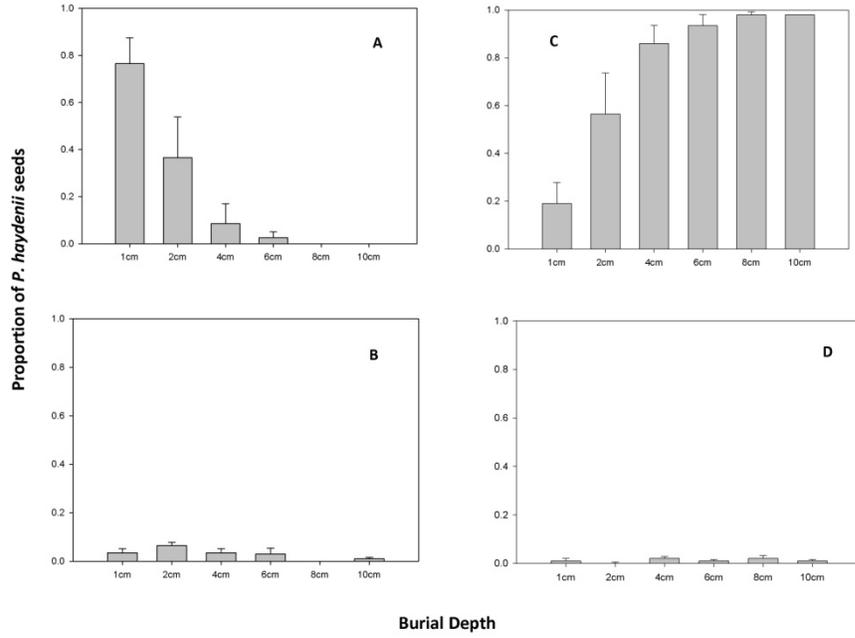


Figure 2

