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Seed Production and Germination of *Penstemon oklahomensis* Pennell (Plantaginaceae), a Southern Great Plains Endemic

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ABSTRACT We investigated the seed production and germination requirements of *Penstemon oklahomensis*, a species of conservation concern, to determine interannual variation in seed production and germination requirements. Mature seed capsules were collected and seed numbers counted in two successive years to assess interannual variation in seed production. A significant difference in number of seeds produced was found between collection years. Germination trials were conducted using the seed collected and subjected to three stratification treatments in two soil types. Seeds held over from the first collection year were also germinated to investigate viability loss a year after collection. Survival analysis was used to estimate the probability of seed germination under the treatment conditions. Germination probability was highest for seeds that underwent a cold stratification period of 60 days if sown the same year as they were collected, regardless of soil type used. Seeds held over a year had higher germination probability after a 30-day cold stratification period, but the probability was lower than the same treatment when the seeds were sown during the year collected. Seed production and germination requirements for *P. oklahomensis* are similar to other species of *Penstemon*, both in the Intermountain West and east of the Mississippi River.
Key words: Germination, *Penstemon oklahomensis*, seed production, survival analysis.

INTRODUCTION The structure and dynamics of spermatophyte populations are a product of the viability, longevity, persistence, and numbers of seeds produced (Fenner and Thompson 2005, Gurevitch et al. 2006), which, for conservation and population restoration purposes, necessitates an understanding of the interannual variation in seed production and dormancy (Baskin and Baskin 2001). Seed dormancy is a condition that facilitates the persistence of a population during dispersal or when faced with adverse environmental conditions, such as drought or temperature extremes. Seeds may exhibit physical or physiological dormancy, or both. Seeds adapted for physical dormancy have hard seed coats that must be abraded either chemically or manually to stimulate germination, whereas physiologically dormant seeds have thinner seed coats and dormancy is broken after a moist cold stratification period (Baskin and Baskin 2004, Fenner and Thompson 2005, Hilhorst 2011). The seeds of most perennial forbs are dormant at release and germinate the following year in late winter or early spring (Baskin and Baskin 2001).

The number of seeds produced by individuals of a species is a measure of reproductive output or effort (Fenner 1985, Tepedino et al. 2006, Zorn-Arnold and Howe 2007, Mabry 2011). Small-seeded species tend to produce higher numbers of seeds while large-seeded species produce fewer seeds (Fenner 1985). Patterns of interannual variability in seed production can be related to climatic variation (Forget et al. 2005) or population size/density (Zorn-Arnold and Howe 2007). During droughts, seed production in

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perennial species may decrease, which could result in a decline in recruitment due to either enhanced seed predation pressure or variability of seed ripened under stress or both (Forget et al. 2005). This situation can be exacerbated by prolonged drought, which could result in the senescence of adult plants and reduced seedling establishment. Low population density can also result in fewer seeds produced per plant if a species is not self-compatible or male and female floral parts are separated in space or time (Zorn-Arnold and Howe 2007). A three-year study determined that *Penstemon tubiflorus* Nutt. capsules contained from 65 to 79 seeds (Mabry 2011). Clements et al. (2002) found no significant differences in the number of seeds per capsule at different capsule heights in a study of *Penstemon hirsutus* (L.) Willd. and *Penstemon tenuiflorus* Pennell; however, significant differences existed in the number of seeds left in a capsule when capsules were harvested two weeks apart for both species.

Seed germination in the genus *Penstemon* has been examined extensively, due in part to the popularity of these plants in horticulture, and has involved several species (Bennett 1981, Nold 1999, Lindgren and Wilde 2003, Diboll 2008, Swayne 2012). The most extensive studies are for species from the Intermountain region of the western United States, which is the center of diversity for the genus (Wolfe et al. 2006). The importance of cold stratification is evident for all species studied, although the extent varies with species. Germination percentages increased for 16 *Penstemon* species following a period of cold stratification; however, the necessary duration of treatment varied by species from 2 weeks to 16 weeks (Kitchen and Meyer 1991). Comparable results were reported for 38 species of *Penstemon* (Allen and Meyer 1990, Meyer et al. 1995), although in the latter case, after-ripened seeds had higher germination than freshly collected seeds. Less than 15% of *Penstemon eatonii* A. Gray seeds germinated without a cold stratification treatment (Meyer 1992).

Lindgren and Schaaf (2004) found that eight species of *Penstemon*, three of which (*Penstemon angustifolius* Nutt. ex Pursh, *Penstemon digitalis* Nutt. ex Sims, and *Penstemon grandiflorus* Nutt.) are allopatric with *P. oklahomensis* Pennell, showed higher germination percentages with two-year-old seed than one-year-old seed. Germination percentages increased for *P. angustifolius* and *P. grandiflorus* as the number of weeks of stratification were increased up to 10 weeks for both one- and two-year-old seeds. However, *P. digitalis* only showed an increase in germination up to eight weeks for one-year-old seed and six weeks for two-year-old seed (Lindgren and Schaaf 2004).

The spring germination percentages for *P. hirsutus* and *P. tenuiflorus*, both from the eastern United States, were 40% or higher, when seeds of each were sown in the previous fall and left in an unheated greenhouse (Clements et al. 2002). In the first year, germination percentages for both species peaked in March of the year following sowing. In the second year, both species had peak germination in April of the year following sowing. Each species had lower germination percentages, between 0% and 30%, in the fall following initial seed harvest. *Penstemon hirsutus* had higher germination percentages in year one, while *P. tenuiflorus* had higher germination percentages in year two, suggesting that although most seeds were dormant at the time of dispersal, some were not (Clements et al. 2002).

Penstemon oklahomensis Pennell is a perennial that flowers from April until June and produces ovate-acuminate capsules that mature and dehisce in late summer or early fall (Pennell 1935). Seeds in the genus *Penstemon* are tan, brown, or black and lack specialized dispersal structures (Freeman In prep). There are no specific studies of seed production and/or germination requirements for *P. oklahomensis*. This species typically occurs in loam or sandy loam soil (Messick and Hoagland 2012) but can also be found in dry clay soils (Pennell 1935). *Penstemon oklahomensis* is most frequently found in remnant tallgrass prairie (Pennell 1935, Messick and Hoagland 2012) however, land use change is causing these habitats to disappear.

The objective of this study was to examine the seed production and germination requirements of *P. oklahomensis* Pennell (Plantaginaceae), a regional endemic of the southern Great Plains. Populations of *P. oklahomensis* occur in 26 counties in Oklahoma (Messick and Hoagland 2013) and a single location in northeastern Texas (Holmes et al. 2010) and has a conservation rank of G3 (Oklahoma Biological Survey 2012). We addressed the following questions, regarding seed production: Does capsule position on the

plant affect the number of seeds produced per capsule? Can capsule dimensions be used to estimate the number of seeds produced per capsule? What is the average number of seeds produced annually by *P. oklahomensis*? Regarding germination, we ask: Are germination rates affected by cold stratification, and if so, does short or longer cold exposure increase germination rates? Do *P. oklahomensis* seeds germinate at higher percentages in greenhouse soil or native soil? Do germination rates decline in the second year beyond harvesting?

MATERIALS AND METHODS In each of two consecutive summers, 2010 and 2011, seed capsules were harvested twice, one week apart, from 30 individual *P. oklahomensis* plants in a naturally occurring population in Norman, Oklahoma (35°11′N, 97°26′W). Only individual plants with six or more capsules present were included in the study. Three capsules were collected from each plant, one each from height categories designated as low, middle, and high. An additional set of capsules was collected one week later in the same manner. Capsules were considered mature and ready for harvest at the first sign of dehiscence, which began in mid-July. Prior to seed removal, the length and width of each capsule was measured. The capsule was then opened and the number of seeds counted. All seeds were then pooled and randomly sorted into lots of 100 seeds for the germination trials. Seeds collected in 2010 that were not used in the first year germination trials were held in the dark at room temperature for use the next year.

The germination trials were conducted inside a greenhouse. Seeds were divided among two soil types, greenhouse soil and native soil, with three stratification treatments: no cold stratification, 30 days cold stratification, and 60 days cold stratification. We tested germination in two soil types: a sandy loam typical of natural conditions (Messick and Hoagland 2012) and a potting mixture, given that plants are often cultivated for population restoration. The native soil was collected from a site near a known population of *P. oklahomensis*, but not from within the population area. The potting mix consisted of two parts peat, one part loam, one part vermiculite, one part perlite, hydrated lime to adjust pH to 6.5, and osmocote fertilizer (14-14-14; Lemke, pers. comm. 2010). The greenhouse was heated in winter so that the inside temperature remained above 10°C. Greenhouse temperature was recorded on days when seed trays were watered. Once the inside temperature exceeded 35°C, the heat was turned off and the air conditioning turned on, during which the temperature would vary between 18°C and 35°C.

For each of the stratification treatments, there were three replicates of 100 seeds for each of the two soil types (potting mix and native soil). All trays were filled with soil to a depth of approximately 2.50 cm and lightly moistened prior to seeding. A 20×28 cm grid template of 10 evenly spaced rows of 10 holes facilitated placement of the seeds in each tray. Once seeded, each tray was lightly misted with water to ensure seed contact with the soil. The no-cold-stratification treatment trays were placed inside the greenhouse and received supplemental water every other day, at which time observations were made for germination.

Trays designated for the 60-day cold stratification were prepared as above and placed in a refrigerator at 5.56°C for the stratification period. Thirty days into this stratification period, the trays for the 30 day cold stratification were prepared and placed in the same refrigerator at the same temperature. While inside the refrigerator, the trays were checked every other day for germination and lightly misted as needed to prevent the soil from drying. Once stratification was complete, all trays were moved to the greenhouse and followed the same watering and observation schedule as the tray of non-stratified seeds.

During summer 2011, a new set of 30 individual plants was selected following the same criteria as the previous year. New individuals were selected because mowing had occurred during the winter months, making relocation of individuals from the previous year impossible. Seed capsule harvest and measurements, seed counting, and seed sorting were repeated as in 2010. Germination trials of the 2011 seeds were conducted exactly as the prior germination trials. The 2010 seeds held over were used in a second year germination trial to assess viability the second year after production.

Once the first cotyledons were visible at the soil surface, a seed was determined to have germinated. As seed germination occurred in all treatments, the total number of dicot seedlings was counted and allowed to grow. If previously counted dicot seedlings were later determined to not be *P. oklahomensis*, they were removed

from the tray and germination counts were adjusted accordingly. Any monocot seedlings that germinated were removed immediately.

Statistical Analyses

Seed production count data were analyzed in two ways. Firstly, paired t tests assessed differences between seed collection weeks within each study year only. Second, an analysis of variance (ANOVA) was used to test for differences in seed counts at the three different heights on the infructescence within each collection year and between collection weeks. A multiple linear regression was then used to test the relationships between seed count and capsule length and diameter.

Seed germination data were analyzed using survival analysis following the guidelines of McNair et al. (2012), with modifications recommended by Kleinbaum and Klein (2012) and Machin et al. (2006), using the Survival Analysis Library v.2.37-7 (Therneau 2014). Survival analysis of germination data tests the failure rate, or hazard rate, of a seed to "not germinate" and produces a survival estimate called the Kaplan-Meier (KM) survival curve. The longer a seed survives as a seed, the lower its survival rate; in terms of germination, the longer a seed stays a seed, its probability of germination increases until a certain time, then the probability of germinating levels off or declines (McNair et al. 2012).

Kaplan-Meier survival curves are then compared statistically using the semiparametric Cox proportional hazards (PH) model, which estimates the proportional hazard for the event to *not* occur. In order to use this test, however, the assumption that the proportional hazard ratio between subjects is the same must be tested by plotting the log-log of the KM survival estimates. If the plotted lines are relatively parallel then the assumption is not violated; however, if the lines are not parallel then the assumption is violated and the appropriate model to run is the stratified Cox PH model. The stratified Cox PH model divides the data into groups based on the explanatory variable that caused the proportional hazard violation (Kleinbaum and Klein 2012, McNair et al. 2012). The stratified Cox PH model was used because our data violated the proportional hazard assumption. The variable that caused the violation was cold stratification treatment. From the stratified Cox PH models, a hazard ratio of 1.00 indicates that there is no relationship or equal probability of the event occurring, which in this study means an equal probability of germination. If the hazard ratio is greater than 1.00, then the first group in the comparison has that many times greater probability of germinating. However, if the hazard ratio is less than 1.00, the first group has that many times less probability of germinating (McNair et al. 2012). All statistical analyses used R v.3.1.1 (R Development Core Team 2014).

RESULTS

Seed Counts and Capsule Dimensions

In 2010, a total of 6,613 seeds were collected from 180 capsules and a total of 15,880 seeds were collected from 180 capsules in 2011. Total seed production was significantly different between the collection years ($t = -10.70$, $p < 0.0001$). The mean number of seeds per capsule was 37 seeds (range 0–190, SD = 37.15) in 2010 and was 88 seeds (range 0–222, SD = 53.07) in 2011. Seeds collected in 2010 had a mean length of 7.67 mm (range 3.45–10.88 mm; SD = 1.56 mm) and diameter of 3.84 mm (range 1.41–5.91 mm; SD = 0.97 mm), while seeds collected in 2011 had a mean length of 7.21 mm (range 2.78–10.44 mm; SD = 1.36 mm) and diameter of 4.42 mm (range 1.93–6.23 mm; SD = 0.90 mm). The number of seeds produced in capsules at different heights on the infructescence were not significantly different whether collected in the same week or between weeks for both years (2010: $F = 2.03$, $p = 0.08$; 2011: $F = 0.21$, $p = 0.96$; Figure 1). Multiple linear regression found a positive relationship between the number of seeds produced and both capsule length and diameter (2010: $r = 0.63$, $R^2 = 0.63$, $p < 0.001$; 2011: $r = 0.80$, $R^2 = 0.65$, $p < 0.001$; Figure 2).

Germination Trials

First year germination: 2010 and 2011 seeds. Germination for the 2010 seeds began 39 days after sowing for the nonstratified seeds. Seeds stratified for 60 days began germinating 8 and 14 days after the end of stratification in native and greenhouse soil, respectively. Seeds stratified for 30 days began germinating 14 and 24 days after stratification in native and greenhouse soil, respectively. The treatment group with the highest germination percentage (35.33%) was native soil stratified for 60 days and the lowest (6.67%) was the native soil with no stratification (Table 1).

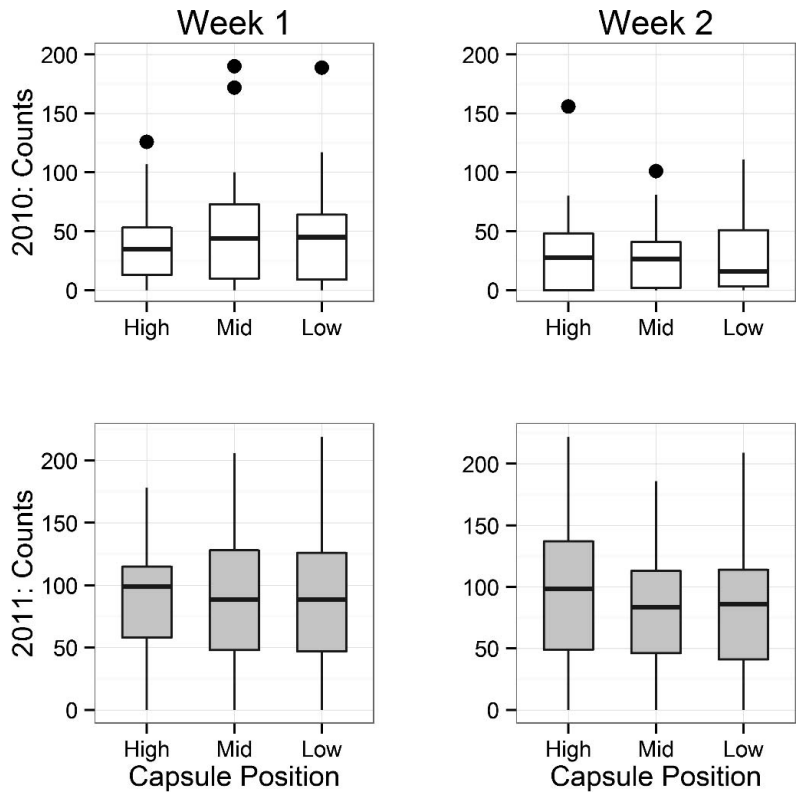

Figure 1. Seeds per capsule in *Penstemon oklahomensis* for 2010 and 2011 per collection week and height of the capsule on the infructescence. High–2010: $F = 0.54$, $p = 0.46$; 2011: $F = 0.48$, $p = 0.49$. Mid–2010: $F = 4.01$, $p = 0.05$; 2011: $F = 0.12$, $p = 0.73$. Low–2010: $F = 4.40$, $p = 0.04$; 2011: $F = 0.33$, $p = 0.57$.

The 2011 nonstratified seeds began germinating 43 and 47 days after sowing in greenhouse and native soil, respectively. Seeds stratified for 60 days began germinating in greenhouse soil 10 days after the end of stratification and in native soil eight days after the end of stratification. Seeds stratified for 30 days began germinating 10 days after the end of stratification for both soils. Overall, more seeds germinated in greenhouse soil than in native soil, regardless of stratification treatment. Seeds receiving a stratification period of 30 days in greenhouse soil had the highest germination percentage with 62.00% while those receiving no stratification in native soil germinated at 1.67% (Table 1).

Second year germination: 2010 seeds. Nonstratified seeds began germinating after 41 days; however, the total number was lower than during the first year germination trials and peak germination occurred 12 days later in the following month. Similar to the first year germination trials, seeds stratified for 60 days began germinating 8 and 12 days after the end of stratification in native and greenhouse soil, respectively. Seeds stratified for 30 days in greenhouse soil began germinating 10 days after the end of stratification, while those in native soil began germinating 12 days after stratification. The highest germination percentage was for the 30-day stratification in greenhouse soil at 33.67% and the lowest was for the 30-day stratification in native soil at 3.67% (Table 1).

Survival Analysis

Kaplan-Meier survival estimates for the seed germination trial year by soil type for each stratification treatment are presented in Figure 3. Recall that these survival estimates represent the probability of the seed surviving as a seed rather than the probability of germination. When sown in either soil type, first year germination of the 2010 seeds was highest (179) after the 60-day stratification period. Conversely, the 2010 seeds

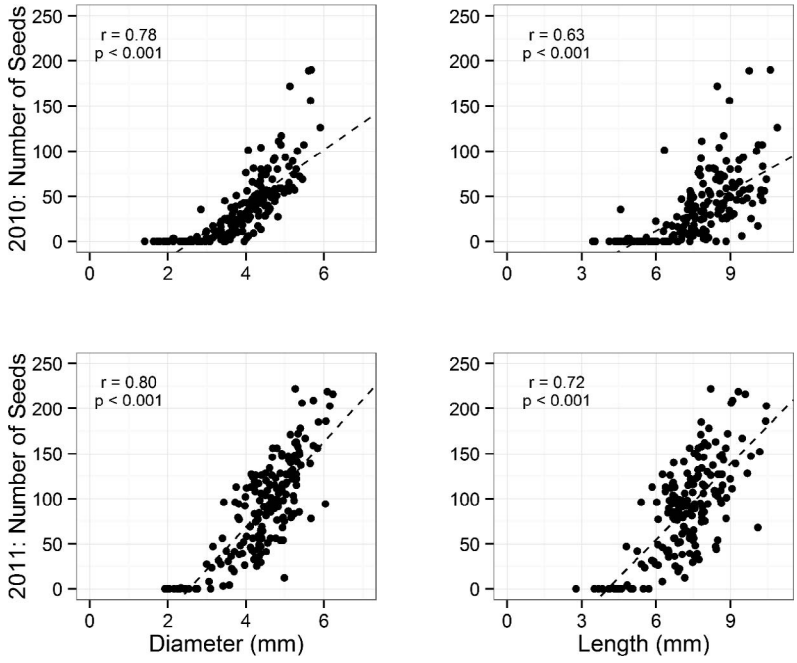

Figure 2. Correlation between number of seeds in a capsule and capsule dimensions in *Penstemon oklahomensis*.

Table 1. Monthly germination numbers for seeds of *Penstemon oklahomensis* grouped by treatment and year. For each treatment, n = 300 and for each seed lot, n = 2,400. GS = greenhouse soil and NS = native soil.

Treatment	Oct	Nov	Dec	Jan	Feb	Mar	Total no.	Total %
2010 seed lot year 1								
GS-I	23	0	0	1	0	0	24	8.00
NS-I	15	2	2	1	0	0	20	6.67
GS-60D	0	0	63	10	0	0	73	24.33
NS-60D	0	0	57	51	0	0	106	35.33
GS-30D	0	0	6	42	4	0	50	16.67
NS-30D	0	0	45	37	0	0	82	27.33
Total	44	2	173	142	4	0	361	15.04
2010 seed lot year 2								
GS-I-2010	4	29	0	4	0	0	37	12.33
NS-I-2010	1	10	4	0	1	5	21	7.00
GS-60D-2010	0	0	15	2	0	0	17	5.67
NS-60D-2010	0	0	43	0	3	0	46	15.33
GS-30D-2010	0	0	90	10	1	0	101	33.67
NS-30D-2010	0	0	11	0	0	0	11	3.67
Total	5	39	165	16	5	5	335	13.96
2011 seed lot year 1								
GS-I-2011	3	14	0	0	0	0	17	5.67
NS-I-2011	1	3	1	0	0	0	5	1.67
GS-60D-2011	0	0	90	15	6	0	111	37.00
NS-60D-2011	0	0	25	1	0	0	26	8.67
GS-30D-2011	0	0	164	10	12	0	186	62.00
NS-30D-2011	0	0	24	1	0	0	25	8.33
Total	5	17	304	27	18	0	371	15.46

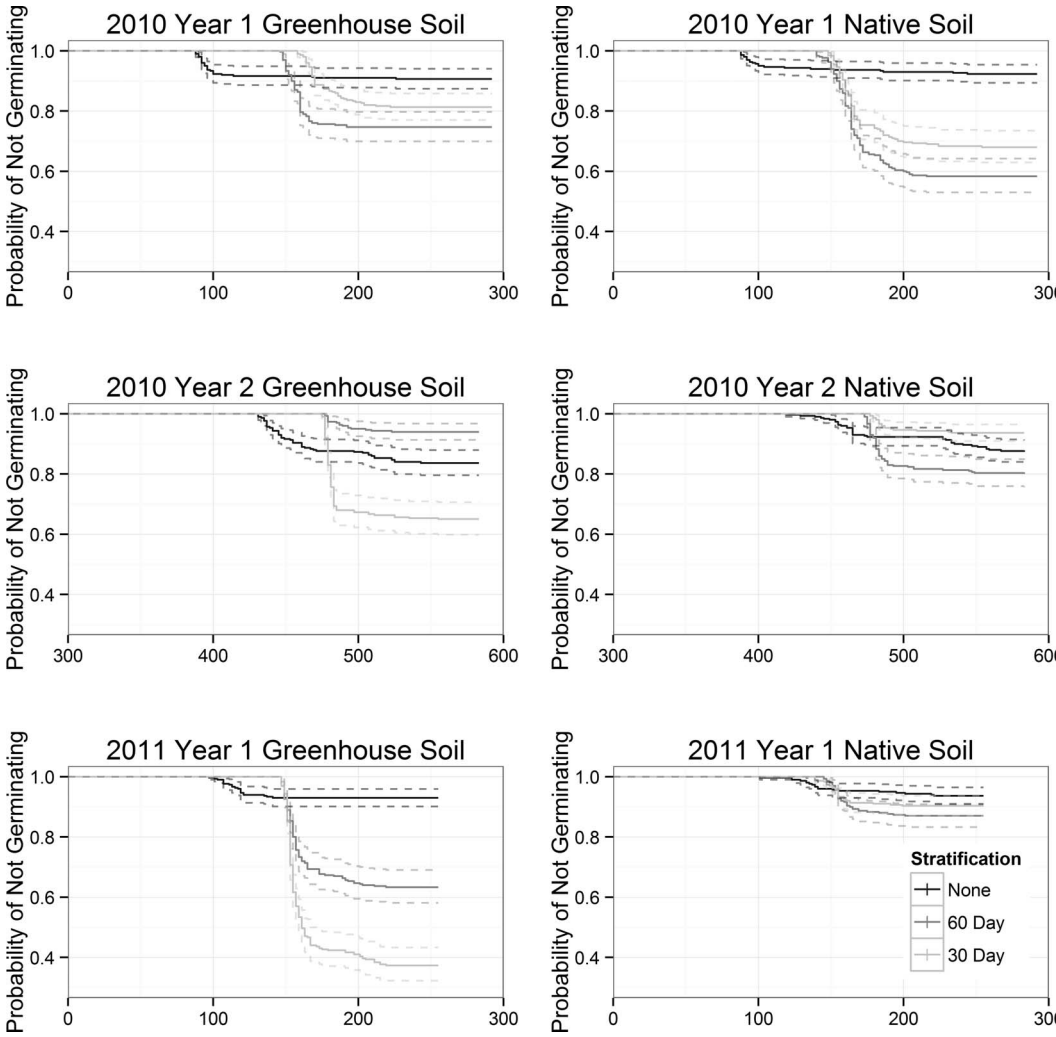

Figure 3. Kaplan-Meier survival estimates by soil type and stratification treatment with 95% confidence intervals (shown with dashed lines). Year 1 = first year germination of seed; Year 2 = seed held over and germinated in second year.

had the lowest germination (17) after the 60-day stratification period when sown in greenhouse soil during the second year, but higher germination (101) after 30 days stratification for the greenhouse soil type. When sown in native soil, the second year germination of the 2010 seeds was highest after the 60-day stratification period (46). The first year germination of the 2011 seeds had the highest germination when sown in greenhouse soil and stratified for 30 days (186). The 2011 seeds in greenhouse soil had the highest germination after the 60-day stratification (111) period (Figure 3).

Pooling soil types, the KM survival curves estimate the probability of the seed not germinating (the probability of surviving as a seed); thus, to determine the probability of germination, the probability of not germinating was subtracted from 1.00 (Figure 4). The survival curve difference tests found that all KM survival curves were significantly different from one another regardless of treatment, soil type, or year (Tables 2 and 3). These tests do not identify which survival curves are different from the others, however, but only show that there is a difference between the survival curves.

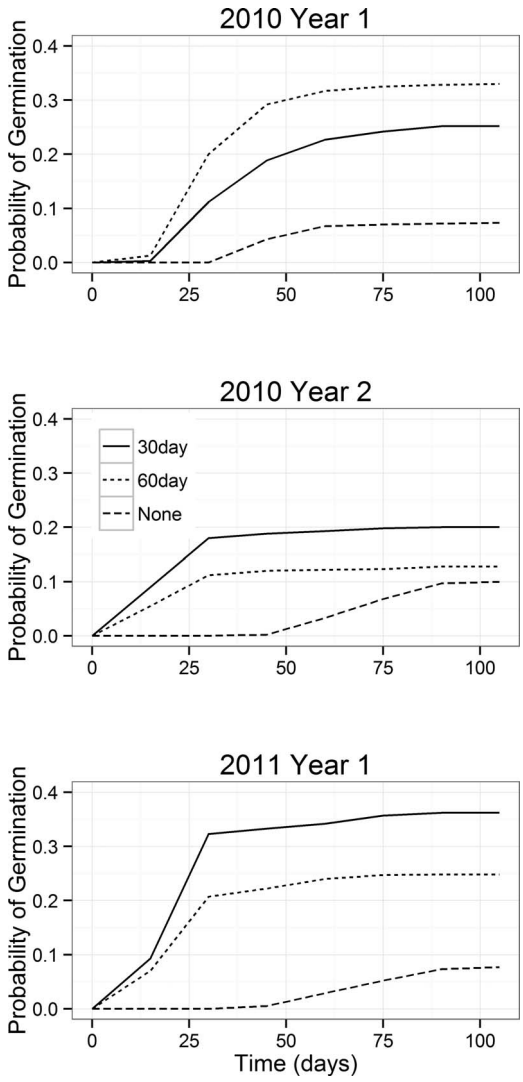

Figure 4. Germination curves by stratification treatment, soil types combined. Year 1 = first year germination of seed; Year 2 = seed held over and germinated in second year.

The stratified Cox PH model hazard ratios allowed identification of which survival curves are different from one another. In our analysis of within year differences, the only nonsignificant comparison was between 2010 seeds stratified for 30 days in greenhouse soil and seeds stratified for 60 days in native soil for the first year germination. All subsequent comparisons within the same germination year were found to be significant (Table 4). We applied a simple Bonferroni correction for the between-year comparisons due to the total number of comparisons made. For this portion of the analysis, we set $\alpha = 0.003$ ($\alpha = 0.05/15$). Of the 2010 seeds, there was not a significant difference between first- and second-year germination, meaning freshly collected seeds from that year were 1.76 times more likely to germinate in the same year than those sown a year later. However, nearly all comparisons for both stratification periods were found to be significantly different between years. Thus, first year germination of the 2010 seeds was 5.32 times more likely to germinate than the 2011 seeds after 30 days stratification and 1.68 times more likely to germinate after 60 days of stratification. In the case of held-over seeds, those from 2010 seeds with first year germination after 30 days of stratification was 2.45 times more likely than holding the seed for one year. Also, the held-over seeds were 0.22 times less likely to germinate after a 60-day stratification period when compared to germination during the year of collection (Table 5).

To assess overall germination per year, KM survival estimates were calculated pooling both soil types and stratification treatments. The total survival/germination probability for both seed years was similar when the seed was sown in the same year as collected. The second year survival/germination probability of the 2010 seed was lower than the first year germination for either collection year. These survival estimates were converted into germination probabilities as described above (Figure 5).

DISCUSSION

Penstemon oklahomensis shares many of the same seed production and germination traits of its congeners. For example, seed production was not significantly different for capsules at different heights on the infructescence. Clements et al. (2002) reported the same results for *P. hirsutus* and *P. tenuiflorus* and suggested this indicated adequate pollination of flowers at all heights. Although we found a positive relationship between the number of seeds per capsule and the size of *P. oklahomensis* capsules, width was the strongest predictor of seed number in both years. We were unable to successfully apply the seed estimation technique of Tependino et al. (2006), developed for the federally listed *Penstemon haydenii* S. Wats.

Intra-annual and interannual seed production show distinct patterns. The numbers of seeds per capsule in each collection week were not

Table 2. Survival curve difference test for seeds of *Penstemon oklahomensis* within year. O = observed, E = expected, and V = variance.

Stratification	n	Observed	Expected	(O−E)^2/E	(O−E)^2/V
2010 seeds year 1 germination					
None	600	41.80	135.00	64.00	117.08
30 days	600	134.90	118.00	2.52	4.23
60 days	600	184.00	108.00	52.79	85.17
				chi sq = 136; df = 2; $p < 0.0001$	
Combination					
GS-none	300	23.10	67.10	28.94	40.45
NS-none	300	18.80	67.50	35.20	49.31
GS-30 days	300	48.20	62.30	3.20	4.36
NS-30 days	300	86.70	55.40	17.72	23.55
GS-60 days	300	71.50	55.50	4.61	6.14
NS-60 days	300	112.50	52.90	67.27	88.52
				chi sq = 178; df = 5; $p < 0.0001$	
2010 second year germination					
None	600	75.30	108.00	9.93	20.77
30 days	600	117.80	77.70	20.67	32.75
60 days	600	73.90	81.20	0.66	1.07
				chi sq = 36.5; df = 2; $p < 0.0001$	
Combination					
GS-none	300	42.80	53.30	2.09	2.99
NS-none	300	32.50	54.70	9.01	13.14
GS-30 days	300	100.40	34.80	123.64	155.73
NS-30 days	300	17.30	42.90	15.20	19.97
GS-60 days	300	16.90	42.50	15.47	20.28
NS-60 days	300	57.00	38.70	8.67	11.14
				chi sq = 191; df = 5; $p < 0.0001$	
2011 seeds first year germination					
None	600	31.70	137.00	80.96	150.12
30 days	600	197.40	109.00	70.97	115.12
60 days	600	134.70	117.00	2.53	4.24
				chi sq = 178; df = 2; $p < 0.0001$	
Combination					
GS-none	300	16.80	68.30	38.80	54.70
NS-none	300	14.90	68.70	42.20	59.60
GS-30 days	300	170.70	46.20	335.80	432.90
NS-30 days	300	26.70	63.10	21.00	28.90
GS-60 days	300	99.60	54.70	36.80	48.90
NS-60 days	300	35.10	62.70	12.20	16.70
				chi sq = 553; df = 5; $p < 0.0001$	

significantly different for both years of the study. This pattern was also reported for *P. hirsutus* and *P. tenuiflorus* (Clements et al. 2002). Seed production, however, was 2.4 times greater in Year 2 than Year 1. This is contrary to Clements et al. (2002), who reported consistent seed production between the years 1992–1993 for *P. hirsutus* and *P. tenuiflorus*. Although there are not comparable studies that repeat seed collection for *P. oklahomensis*, we posit this could be attributable to extreme drought conditions that developed during the flowering period in Year 2 of the study, whereas total precipitation was near normal in Year 1 (Artusa 2012).

Penstemon oklahomensis seeds appear to have an after-ripening period once dispersed from the parent plant as well, as a cold stratification requirement. Our findings are consistent with other species of *Penstemon*, both in the Intermountain region and east of the Mississippi River in the United States (Allen and Meyer 1990, Kitchen and Meyer 1991, Meyer et al. 1995, Clements et al. 2002). When sown in the year following collection, nonstratified seeds germinate at a slightly higher rate than the previous year, suggesting an after-ripening period. Again, this is consistent with other species of *Penstemon* (Allen and Meyer 1990, Clements et al. 2002, Lindgren and Schaaf 2004).

Germination was affected by soil type, with more seeds germinating in the potting mix than in native soil. This has implications in future

Table 3. Survival curve difference test results for seeds of *Penstemon oklahomensis* between years. O = observed, E = expected, and V = variance.

Year	Soil	n	Observed	Expected	(O–E)^2/E	(O–E)^2/V
No stratification treatment						
2010A	GS	300	27.40	27.60	0.001	0.001
	NS	300	22.30	28.20	1.24	1.57
2010B	GS	300	46.50	27.50	13.10	16.39
	NS	300	35.40	28.60	1.62	2.06
2011A	GS	300	20.20	28.50	2.41	3.04
	NS	300	17.70	29.10	4.47	5.68
					chi sq = 24; df = 5; p = 0.0002	
30 day stratification treatment						
2010A	GS	300	43.70	79.80	16.36	23.47
	NS	300	78.10	75.10	0.12	0.17
2010B	GS	300	98.00	63.20	19.18	26.06
	NS	300	16.00	80.10	51.34	73.79
2011A	GS	300	168.20	54.90	233.55	308.39
	NS	300	26.40	77.20	33.50	47.70
					chi sq = 411; df = 5; p < 0.0001	
60 day stratification treatment						
2010A	GS	300	66.60	64.90	0.05	0.06
	NS	300	105.80	63.00	29.15	39.80
2010B	GS	300	16.20	68.60	39.95	55.68
	NS	300	55.90	61.70	0.55	0.75
2011A	GS	300	100.30	56.30	34.38	45.76
	NS	300	35.40	65.80	14.04	19.37
					chi sq = 135; df = 5; p < 0.0001	

restoration efforts and recruitment of new individuals within a population. Lower numbers of seedlings would be expected in the event of direct seeding of an area selected for restoration. Seeds sown in a potting mix and grown out prior to planting would have higher germination success, but transplant survival success cannot be known from our study and should be evaluated.

Table 4. Hazard ratios from stratified Cox proportional hazard models for seeds of *Penstemon oklahomensis*, within year. GS = greenhouse soil, NS = native soil, none = no stratification, 30days = 30 days cold stratification, 60days = 60 days cold stratification. Bolded p-values indicate significant outcomes (α = 0.05).

Year	Comparison	Hazard Ratio	p-value
2010A	30days–60days	1.53	**0.0155**
	30days–none	0.43	**0.0004**
	GS–NS	1.95	**< 0.0001**
	30days:GS–60days:NS	0.90	0.6211
	30days:GS–none:NS	0.42	**0.0079**
2010B	30days–60days	0.14	**< 0.0001**
	30days–none	0.26	**< 0.0001**
	GS–NS	0.14	**< 0.0001**
	30days:GS–60days:NS	26.02	**< 0.0001**
	30days:GS–none:NS	5.29	**< 0.0001**
2011A	30days–60days	0.47	**< 0.0001**
	30days–none	0.06	**< 0.0001**
	GS–NS	0.10	**< 0.0001**
	30days:GS–60days:NS	2.91	**< 0.0001**
	30days:GS–none:NS	8.71	**< 0.0001**

Germination rates were lower in the second year after seed harvest that the first. This could be due to seed viability loss over time for the older seed. The first year germination rate of the 2011 seed lot was significantly higher than the second year germination of the 2010 seed lot for both cold stratification treatments (Table 5).

In conclusion, *P. oklahomensis* germination is enhanced by cold stratification, a trait shared by its congeners. The germination timing and percentages found here indicate *P. oklahomensis* seeds are released from the parent plant in various states of dormancy. A proportion of the seeds were only partially dormant and required after-ripening before breaking dormancy. These seeds benefit from a cold stratification period of 30–60 days, regardless of soil type if sown during the same year as collection. Older seeds do not need a longer stratification period, but rather a relatively short period. This may indicate that *P. oklahomensis* forms a seed bank in its environment. However, Clements et al. (2002) reported that *P. hirsutus* and *P. tenuiflorus* did not form a persistent seed bank in their environments. A proper seed bank study should be conducted

Table 5. Hazard ratios from stratified Cox proportional hazard models for seeds of *Penstemon oklahomensis*, between years. GS = greenhouse soil, NS = native soil, none = no stratification, 30days = 30 days cold stratification, 60days = 60 days cold stratification. Bolded p-values indicate significant differences after Bonferroni correction ($\alpha = 0.003$).

Stratification	Comparison	Hazard Ratio	p-value
None	2010A–2010B	1.76	0.0172
	2010A–2011A	0.73	0.2680
	GS–NS	0.80	0.4356
	2010A:GS–2010B:NS	0.93	0.8318
	2010A:GS–2011A:NS	1.10	0.8206
30 days	2010A–2010B	2.45	< **0.0001**
	2010A–2011A	5.32	< **0.0001**
	GS–NS	1.85	**0.0003**
	2010A:GS–2010B:NS	0.07	< **0.0001**
	2010A:GS–2011A:NS	0.05	< **0.0001**
60 days	2010A–2010B	0.22	< **0.0001**
	2010A–2011A	1.68	**0.0006**
	GS–NS	1.70	**0.0003**
	2010A:GS–2010B:NS	2.20	0.0100
	2010A:GS–2011A:NS	0.17	< **0.0001**

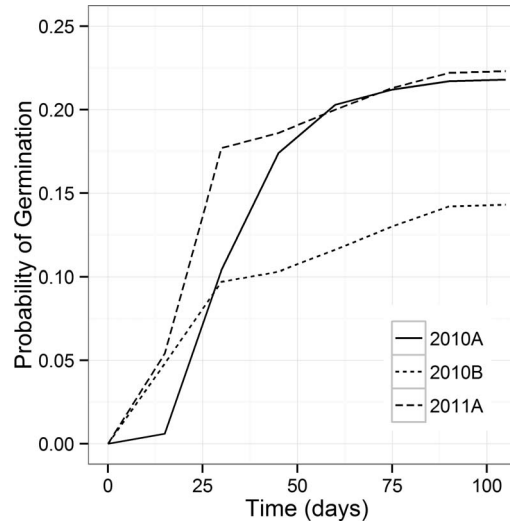

Figure 5. Germination curves by year, all stratification treatments and soil types combined within year. YearA = first year germination of seed; YearB = seed held over and germinated in second year.

with *P. oklahomensis* to confirm this speculation.

A possible consequential finding, relative to the ecology and conservation of this species, is the significant difference in the number of seeds produced between the two collection years. Contrary to the expectation that more seeds are produced with increased precipitation (Evans and Dennehy 2005), *P. oklahomensis* increased seed production during drought. The high number of seeds produced could be an anomalous result, an example of diversified bet-hedging (Childs 2010), or adaptive plasticity (Evans and Dennehy 2005). Either of the latter two could be possible when coupled with the various states of dormancy upon release from the parent plant found in our study. Since this study and that of Clements et al. (2002) were only two years in duration, we suggest a long-term seed production study to answer this question. Although the specific reasons for this difference were not investigated directly, we speculate it was possibly related to the drought. Thus, there could be an inverse relationship between seed production and precipitation during flowering and seed set but more yearly data would be needed to further assess the relationship.

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