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Influence of Seed Stratification and Seed Age on Emergence of *Penstemon*

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***Abstract*. Studies were conducted to evaluate the effect of stratification and seed age on percent seedling emergence of *Penstemon*. Emergence differences occurred between the eight *Penstemon* selections, as well as between seed stratification treatments and seed age. Seed stratification significantly increased emergence. Emergence varied from 0% with 1-year-old seed of *Penstemon digitalis* with no stratification, to 72.8% emergence with 2-year-old seed of *P. angustifolius* with 10 weeks of stratification. Seedlings from 3- to 4-year-old seed generally emerged as well as or better than with 1- and 2-year-old seed. Percent emergence varied significantly with stratification, seed age, and species. Some emergence occurred with species from seed up to 10 years old.**

Growing and producing native plants for commercial, residential, and highway plantings is an important component of the horticulture industry. However, the success of growing native plants from seed can vary greatly with species, seed source, seed treatment, seed age, environment and pre- and postgermination conditions. *Penstemon*, a diverse genus of ≈270 species, is native from Alaska south to North and Central America to Guatemala. The genus is diverse for flowering traits, foliage, plant size and ease of propagation and has a wide variety of uses (Lindgren and Wilde, 2003). Penstemon species have been reported to respond to pre-germination seed treatments including stratification, scarification, alternating temperatures, growth regulators, and light (Allen and Meyer, 1990; Deno, 1991; Kitchen and Meyer, 1991; Lindgren, 1990; Raeber and Lee, 1991; Salac and Hesse, 1975). The response of penstemon seed to pre-germination treatments varies extensively with species. The objective of this study was to evaluate the effect of seed age and cold stratification on the percent seed emergence of a diverse selection of *Penstemon*.

Methods and Materials

Seeds of seven *Penstemon* species (*P. angustifolius* Nutt. ex. Pursh, *P. barbatus* (Cav.) Roth, *P. digitalis* Nutt., *P. gracilis* Nutt., *P. grandiflorus* Nutt., *P. haydenii* S. Wats., *P. strictus* Benth.,) and one hybrid selection, 'Prairie Splendor' (*P. cobaea* Nutt. × *P. triflorus* Heller) were collected from the University of Nebraska West Central Research and Extension Center field research plots located near North Platte. These *Penstemons* represent a wide range of flowering and foliage characteristics, growth habits, and adaptation. All seeds used in the 11 years of this study were collected in 1988 from plants transplanted to field plots in 1985. All seeds were air dried and stored at 16 to 18 °C, at an average of 35% relative humidity until planting. In 1989 and 1990, seeds from each of the eight *Penstemon* selections were stratified (cold and moist) for 0, 2, 4, 6, 8, or 10 weeks. Due to limited amounts of seed, the number of selections included in the 1991 to 1999 studies were reduced to four, and stratification treatments reduced to two treatments, 0 or 8 weeks. The results of the 1989 and 1990 studies are reported together as a short-term study, and the 0- or 8-week stratification treatment of four of the selections (*P. digitalis, P. grandiflorus, P. strictus,* and *P.* × 'Prairie Splendor') for all years, 1989 to 1999, are reported together as a long-term study. Table 1 summarizes the treatments included in the short- and long-term studies.

All seeds in these studies were initially stratified the first week of January, beginning with the longest stratification treatment. Seeds were stratified in Rediearth (W.R. Grace & Co., Cambridge, Mass.) growing substrate in 12.0 L (48 × 34 × 7.5 cm) metal containers. Seeds in all treatments were covered with 2 mm of substrate. All of the seeded containers in the 1989 and 1990 studies for the 0, 2, 4, 6, 8, or 10 week stratification times, were seeded at 14-d intervals for each stratification treatment. They were moved to a greenhouse simultaneously when the 0-week treatment was planted. For the 1991 to 1999 studies, the 8-week stratification treatments were planted the third week in January of each year and moved to the greenhouse 8 weeks later when the 0-week treatment was planted. The substrate with the planted seeds, except for the 0-week treatments, were watered until saturated and placed on the floor in a cold storage facility 24 h after seeding. Substrate temperatures were 1 ± 3 °C during the stratification period. The seeded containers were checked weekly for moisture. The growing substrate was lightly misted as needed, to maintain sufficient substrate moisture. Once in the greenhouse, air temperature was maintained at a minimum of 21 °C during the day and a minimum of 18 °C at night during emergence. No supplemental light was provided. Each species and selection consisted of a 50-seed sample per treatment, and all treatments were replicated four times. Percentage seed emergence for each treatment was recorded weekly for 4 weeks. Emergence was defined as the first sign of a visible seedling. Emergence was reported as the percent of the total number of seedlings, out of 50 seeds planted, that emerged during the 4 weeks. Although emergence is also a function of germination, the results here are reported as emergence. Treatments were analyzed as a split-split-plot design in the short-term experiment with species as the main plot, stratification as the subplot and years as sub-subplots. In the long-term study, which included all years, four selections and two treatment times, seed age was used as the main plot, stratification as subplot and species as sub-subplot for analysis. Data were analyzed using Proc MIXED (SAS, 1989).

Results and Discussion

There were significant differences ($P \leq 0.01$ or 0.001) in emergence among species, weeks of seed stratification and seed age in the short-term (2-year) study (Table 2). There was also a significant interaction for species × stratification treatment (weeks), for age × species and age × weeks × species. Most of the selections included in this 2-year study had greater percent emergence with 2-year-old seed, compared to emergence from 1-year-old (past season's) harvested seed. However, this did not hold true for *P. haydenii*. *Penstemon gracilis* emergence was higher for 2-year-old seed, compared to 1-year-old seed for all of the stratification treatments. For most of the selections, emergence was highest with the 8- to 10-week stratification treatments. Many interactions were present. For example, with *P. strictus,* emergence decreased at the 8- and 10-week stratification treatment for 2-year-old seed but increased at the 8- and 10-week stratification for 1-year-old seed.

Table 1. Summary of *Penstemon* treatment variables.

Parameter	Short-term study	Long-term study
Years in study	2 (years 1 and 2)	11 (years 1 to 11)
Seed stratification treatment (weeks)	0, 2, 4, 6, 8, 10	0, 8
Selections included	*P. angustifolius*	*P. digitalis*
	P. barbatus	*P. grandiflorus*
	P. digitalis	*P. strictus*
	P. gracilis	*P.* 'Prairie Splendor'
	P. grandiflorus	
	P. haydenii	
	P. strictus	
	P. 'Prairie Splendor'	

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Table 2. Emergence (%) of *Penstemon* with zero to 10 weeks of stratification for 1-year old (1) and 2-year old (2) seed and the analysis of variance.

Species/selection		Weeks of stratification 0	2	4	6	8	10
P. angustifolius	(1)	1.5	6.0	4.0	18.0	53.0	68.0
	(2)	9.3	29.8	7.0	38.5	71.3	72.8
P. barbatus	(1)	15.0	8.0	9.0	10.5	13.0	15.0
	(2)	13.5	12.0	15.0	17.0	22.3	26.5
P. digitalis	(1)	0.0	1.0	9.5	13.5	16.5	13.0
	(2)	0.0	13.5	30.5	48.5	25.0	26.5
P. gracilis	(1)	0.5	0.5	7.0	11.0	22.5	33.0
	(2)	29.0	44.0	51.5	60.0	66.5	56.5
P. grandiflorus	(1)	5.5	4.5	8.0	8.0	25.0	24.0
	(2)	11.5	21.5	2.5	17.0	35.5	32.5
P. haydenii	(1)	5.0	4.0	2.5	3.0	4.0	8.5
	(2)	5.5	4.0	2.5	5.5	4.0	2.5
P. strictus	(1)	3.5	5.0	3.5	9.0	27.5	30.0
	(2)	16.0	13.0	15.5	49.0	41.5	13.3
P. 'Prairie Splendor'	(1)	0.0	3.0	1.0	1.0	1.5	1.0
	(2)	1.5	4.0	2.0	5.0	16.0	18.5

Source	df	F value	*P*
Age	1	281.60	***
Weeks	5	88.99	***
Age × weeks	5	5.61	**
Species	7	136.85	***
Age × species	7	37.90	***
Weeks × species	35	14.07	***
Age × weeks × species	35	3.98	***

,*Significant at $P \leq 0.01$ or 0.001 respectively.

Table 3. Percent emergence of four *Penstemon* species/selections of seed 1 to 11 years old.

	'Prairie Splendor'		*P. digitalis*		*P. grandiflorus*		*P. strictus*	
	Stratification (weeks)							
Year	0	8	0	8	0	8	0	8
1	0.0	1.5	0.0	16.5	5.5	25.0	3.5	27.5
2	2.0	1.5	0.0	30.5	13.5	29.0	23.5	47.5
3	5.0	42.5	7.5	36.0	11.5	24.5	42.5	30.0
4	4.0	40.0	5.5	35.0	20.5	29.0	43.5	51.0
5	9.5	0.0	3.5	16.0	7.5	0.5	44.0	29.5
6	8.0	0.0	2.5	13.5	5.0	0.0	28.5	21.5
7	3.0	18.5	1.0	33.5	0.5	2.0	14.0	10.0
8	10.5	3.0	2.0	7.0	1.5	1.0	8.0	4.5
9	7.0	0.5	0.0	1.0	0.0	0.0	1.5	0.0
10	2.5	0.5	0.0	0.0	1.0	0.0	0.0	0.0
11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Source	df	F value	*P*
Age	10	176.44	***
Weeks	1	147.01	***
Age × weeks	10	42.61	***
Species	3	127.67	***
Age × species	30	18.43	***
Weeks × species	3	59.25	***
Age × weeks × species	30	9.02	***

***Significant at $P \leq 0.001$.

For the long-term (11 years) study (Table 3), there were highly significant ($P \leq 0.001$) differences in emergence for seed age, stratification treatments (weeks), species and all interactions. For the four species included in this study, emergence for the 8-week stratification treatment, was good for seed up to 4 years old. Seed emergence improved only slightly with no stratification (0-week treatment) for *P.* 'Prairie Splendor' and *P. digitalis* but was much higher for *P. strictus* and *P. grandiflorus* with the 0-week treatment for seed up to 4 years old. This would suggest that without some stratification, emergence for *P.* 'Prairie Splendor' and *P. digitalis* will be low with any age of seed. *Penstemon grandiflorus* and *P.* 'Prairie Splendor' had some emergence with seed age up to 10 years old.

For *P. strictus*, emergence was considered good for both 0- and 8-week treatments for seed up to 6 years old. This study indicates that 2-year-old and older seed of *P. strictus* does not need a seed stratification treatment to enhance emergence. For 'Prairie Splendor', seedling emergence was quite low over all years and stratification treatments, except for 3- and 4-year-old seed with 8 weeks of stratification. For *P. grandiflorus,* emergence decreased sharply for seed that was >4 years old for both the 0- and 8-week stratification treatments.

These studies suggest a seed stratification treatment significantly enhanced emergence for some *Penstemon* species but not for other species, such as *P. haydenii*. Allen and Meyer (1990) found similar results for *P.* 'Bandera', a selection of *P. strictus. Penstemon* seedlings, from seed that is 2 to 5 years old, may emerge better than from 1-year-old seed because of after ripening (Allen and Meyer, 1990). Other seed treatments, such as seed scarification, have been successful in increasing germination of *P. haydenii* seed (Stubbendieck et al., 1993). Because *P. haydenii* is found in areas of blowing sand, it may have evolved with seed scarification.

It has been reported that light is necessary for the germination of some species such as *P. digitalis* (Deno, 1991). Although this study did not include a variable light treatment, the results would suggest that *P. digitalis* emergence could be enhanced with stratification even without light.

Other factors that may have influenced the results of these studies could include the influence of seed storage temperatures and seed moisture content (Glenn et al., 1998). The source of the seed and seed harvest dates can also influence penstemon germination patterns (Meyer et al., 1995). Evolutionary patterns and dormancy variation among *Penstemon* seeds collected from a single population may be adaptive features in unpredictable environments (Kitchen and Meyer, 1991; Meyer et al., 1995).

Penstemon emergence is influenced by many factors (seed stratification, age of seed, storage conditions, germination temperature, harvesting conditions, etc.) and by an interaction of these factors for breaking seed dormancy (Khan, 1977). Optimum emergence conditions proposed for one *Penstemon* species or cultivar may be quite different for other *Penstemon* species especially for those originating from diverse sources. Germination and emergence standards for *Penstemon* can not be stated as a single numerical recommendation. Each *Penstemon* species needs to be considered separately, when making recommendations in optimizing and in setting standard protocols for emergence and germination.

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