Seed Germination of Intermountain Penstemons as Influenced by Stratification and GA_3 Treatments[1]

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Abstract

Propagation of the genus *Penstemon* for use in landscape horticulture has been handicapped by a lack of understanding of seed dormancy and a practical method for breaking dormancy for numerous species. The extent of dormancy in seeds of 27 wild populations of *Penstemon* representing 16 Intermountain species was investigated by subjecting seeds to stratification (moist prechilling) of 2 to 16 weeks at 2°C (36°F) and varying concentrations of gibberellic acid (GA_3). Germination varied from 0 to 88% for non-treated seeds and from 13 to 100% for seeds treated with 250 ppm GA_3. Collections from 10 species required 12 or more weeks of stratification for complete germination. Three species exhibited reduced germination after stratification when compared to the non-chilled control. Gibberellic acid significantly reduced the stratification requirement of seeds for the more dormant species. Concentrations of 150, 250, and 500 ppm GA_3 were equally effective in breaking dormancy for most species. The considerable variability in seed dormancy suggests that the most practical solution to penstemon propagation from seed may be the selection of species and ecotypes with minimal dormancy.

Index words: dormancy, beard-tongue, wildflower, native plants

Species used in this study: bush penstemon, *Penstemon ambiguus* Torr.; Wasatch penstemon, *Penstemon cyananthus* Hook.; firecracker penstemon, *Penstemon eatonii* Gray; Fremont penstemon, *Penstemon fremontii* Torr. & Gray; low penstemon, *Penstemon humilis* Nutt. ex A. Gray; Zion penstemon, *Penstemon laevis* Pennell; Kolob penstemon, *Penstemon leiophyllus* Pennell; Leonard penstemon, *Penstemon leonardii* Rydb.; thickleaf penstemon, *Penstemon pachyphyllus* Gray; Palmer penstemon, *Penstemon palmeri* Gray; hillside penstemon, *Penstemon platyphyllus* Rydb.; Bridges penstemon, *Penstemon rostriflorus* Kellogg; littlecup penstemon, *Penstemon sepalulus* A. Nels.; Rocky Mountain penstemon, *Penstemon strictus* Benth.; skyline penstemon, *Penstemon subglaber* Rydb.; and Whipple penstemon, *Penstemon whippleanus* A. Gray.

Significance to the Nursery Industry

With increased emphasis on low-maintenance landscape plantings, the need for hardy native species, such as penstemons, will continue to rise. This research identified populations of *Penstemon* species that produce nondormant seeds as well as populations that produce seed requiring extensive chilling periods, GA_3, or both, to break dormancy. These results provide greater understanding of the germination requirements of some *Penstemon* species and thus increase the opportunity for their cost-effective propagation.

Introduction

Penstemon, the largest genus of the family *Scrophulariaceae*, consists of approximately 250 species of perennial herbs and subshrubs. The genus occurs principally in North America, with its greatest diversity in the Western United States. Penstemons are found in all climatic zones within this region and in general prefer full sun on well-drained mineral soils (8).

Several species of penstemon have been evaluated for use in revegetation and low-maintenance landscapes (15, 16, 17, 18), and many species are cultivated in home gardens by penstemon enthusiasts (12). The potential also exists for containerized production by the nursery industry. Thus, seed germination information is needed to obtain uniform propagation. Although research on penstemon seed germination biology has been limited, a number of studies have shown that the seeds of many species are dormant (2, 13, 19).

Seed dormancy functions to postpone germination until a time of minimum risk to seedling survival (9). Many species from temperate latitudes require a period of moist prechill (stratification) to break seed dormancy, thus delaying germination until harsh winter conditions have passed (5). The required duration of chilling necessary to break dormancy is often indicative of the severity of winters in the natural habitat (4).

Because penstemons are found in a wide range of habitats, considerable variation in seed stratification requirement can be expected. For example, Salac and Hesse (17) reported that 3 weeks at 4°C (39°F) broke dormancy in fresh seeds of *Penstemon grandiflorus* Nutt. In contrast, Allen and Meyer (2) found that 8 weeks of stratification at 5°C (41°F) were inadequate for total germination in fresh seed of *P. eatonii* and *P. strictus*. They reported that one year-old *P. eatonii* seed germinated to only 15% after 2 weeks of stratification, while one year-old *P. strictus* had complete germination after the same treatment. Furthermore, their data indicate that stratification may reduce germination for some seed lots of *P. palmeri*. These data are indicative of the variability that exists in the germination biology of various penstemon species.

Gibberellins (GAs) have been successfully used to break seed dormancy in many species (5), including penstemons (3, 13, 17), suggesting that endogenous GAs might play an important role in germination readiness (20). However, ef-

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fective GA concentrations are generally much higher than those found in embryonic tissue (6). The use of GA to overcome seed dormancy may be enhanced by combining it with other treatments such as stratification or scarification.

The genetic diversity of the genus *Penstemon* provides considerable opportunity for selection. Species and ecotypes that possess desirable characteristics need to be identified. Minimal stratification requirement and uniform germination are two such characteristics. Assessing these traits on penstemons from the Intermountain region was the first objective of this research. A second objective was to determine the effectiveness of GA_3 in breaking dormancy in seeds of these same species. Finally the third objective was to determine if germination response to GA_3 can be used to predict stratification requirement.

Materials and Methods

Seed acquistion. During the summer and fall of 1986 mature seed was harvested from 27 wild populations representing 16 species (Table 1). Inert matter and unfilled seeds were removed from each collection using standard cleaning techniques. Seeds were stored in envelopes at approximately 20°C (68°C).

Viability evaluation. Viability for each collection was determined using the tetrazolium (TZ) test. Four replications of 25 seeds were imbibed on blotters overnight at 20°C (68°F). Each seed was then pierced with a needle and placed in a 1% solution of 2,3,5-triphenyl tetrazolium chloride (TTC) for 24 hours. All seeds were then classified as germinable or nongerminable according to established procedures (10).

Table 1. Species, locations, and elevations of the 27 seed collections used in this study.

Species[z]	Lat (N)	Long (W)	Elevation (m)
GROUP I			
P. ambiguus	37° 11′	113° 45′	990
P. pachyphyllus	38° 45′	111° 30′	2,160
P. palmeri (1)	42° 57′	115° 05′	930
(2)	37° 14′	113° 21′	1,050
(3)	39° 42′	111° 45′	1,740
P. rostriflorus	37° 14′	112° 54′	1,740
P. strictus	39° 47′	111° 43′	2,040
GROUP II			
P. leiophyllus	37° 37′	112° 49′	3,000
P. subglaber (1)	40° 19′	111° 15′	2,370
(2)	39° 40′	111° 19′	2,580
(3)	39° 37′	111° 19′	2,880
GROUP III			
P. leonardii (1)	40° 18′	111° 37′	2,040
(2)	39° 48′	111° 42′	2,430
P. platyphyllus	40° 37′	111° 42′	1,890
P. sepalulus (1)	40° 26′	111° 46′	1,560
(2)	40° 21′	111° 32′	1,680
(3)	40° 26′	111° 40′	2,100
GROUP IV			
P. cyananthus (1)	40° 10′	111° 21′	2,160
(2)	40° 24′	111° 27′	2,160
(3)	40° 44′	111° 31′	2,100
P. eatonii (1)	39° 52′	111° 46′	2,460
(2)	40° 21′	111° 32′	1,680
P. fremontii	40° 23′	109° 34′	1,680
P. humilis (1)	40° 10′	111° 21′	2,160
(2)	37° 14′	112° 54′	1,710
P. laevis	37° 14′	112° 54′	1,710
P. whippleanus	40° 41′	110° 57′	3,000

[z]Scientific nomenclature follows Cronquist et al. (8).

Treatments. Samples from all 27 collections were subjected to stratification at 2°C (36°F) for 2, 4, 8, 12, and 16 weeks and stratification in combination with 250 ppm GA_3 for 16 weeks. Unchilled controls with and without GA_3 were also included. In addition, samples of select collections were stratified with GA_3 for 2, 4, 8, and 12 weeks (Fig. 1). Finally, samples from one collection of most species were treated with 50, 150, 250, and 500 ppm GA_3 to assess the effectiveness of various GA_3 concentrations. All treatments were initiated on December 26, 1986.

Experimental procedures. Each of four replications consisted of 25 seeds placed on top of two standard germination blotters in a 100 × 15 mm petri dish. Blotters were moistened to saturation with either distilled water or the appropriate solution of GA_3. Additional water was added as needed to keep the blotters moist.

After imbibition, all dishes assigned stratification treatments were sorted according to block (replication), randomized, and stacked in cardboard boxes. Each box was enclosed in a plastic bag to facilitate handling and to help retain moisture and placed in the dark at 2°C (36°F). At the termination of each stratification period the appropriate dishes were removed from each box, randomized into four new boxes, and placed in a growth chamber at a constant 15°C (59°F) for 28 days. The boxes were opened and the dishes exposed to short periods of fluorescent light as the data were recorded twice weekly. Dishes not assigned a stratification treatment were boxed and handled similarly, with the exception that no resorting of dishes was necessary.

Seeds with radicle extension >1 mm were counted as germinated. Germination percentages were arcsine transformed for statistical analysis. The experimental results of each collection were analyzed separately. Analysis of variance and significant differences among treatments were determined using the Student-Neuman-Keul (SNK) method.

Results and Discussion

At least one treatment for each collection produced a germination percentage not significantly different from total viability as determined by the TZ test. In order to clarify the presentation of data, all germination percentages have been adjusted for total viability using the highest germination percentage as an estimate of 100% viability.

Species were grouped based upon initial dormancy and response to the various treatments. The seven least dormant collections (five species) had a mean germination of 71% for the control and were assigned to group I (Table 2). The remaining 20 collections (11 species) had minimal germination (<15%) in the control and were divided into groups based upon their response to stratification and GA_3.

Group I. Seed of the *P. strictus* and one of the *P. palmeri* collections were determined to be nondormant and showed no treatment response. The germination responses to chilling for the other five collections varied considerably (Table 2). Seed of the *P. rostriflorus* collection had nearly complete germination after 4 weeks of stratification. Germination for the collection of *P. ambiguus* was strongly inhibited by all stratification treatments. Similarly, the *P. pachyphyllus* and

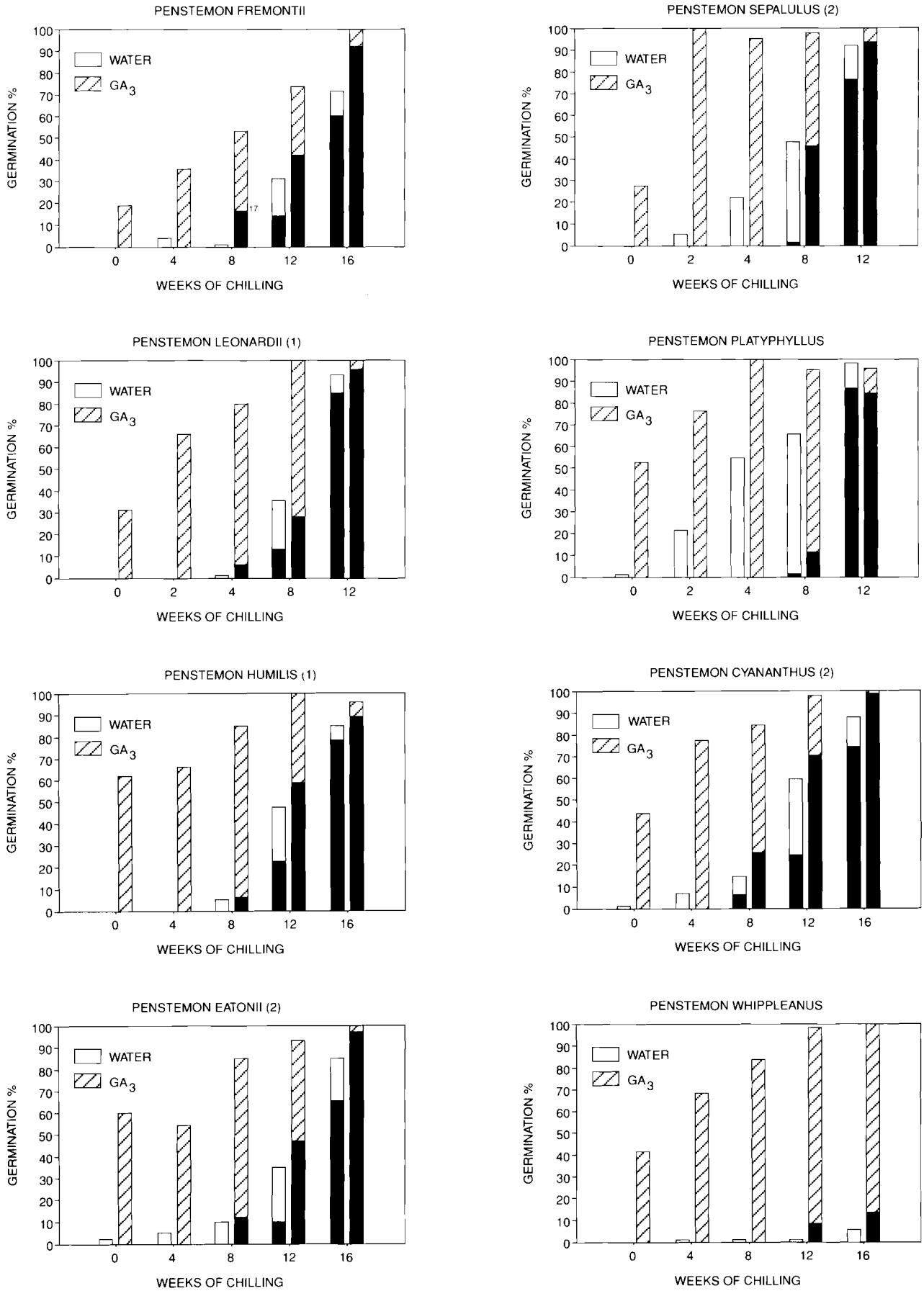

Fig. 1. Germination of 8 collections of *Penstemon* seed after various periods of stratification on blotters containing water only or 250 ppm GA_3. Solid bars indicate the percentage of seeds that germinated during the cold treatment.

Table 2. Germination percentages[z] as influenced by varying periods of stratification and treatment with GA_3.

Collection	Weeks of Stratification 0	2	4	8	12	16	GA_3
GROUP I							
P. ambiguus	47 b	26 c	16 cd	4 e	11 de	7 de	100 a
P. pachyphyllus	71 b	47 cd	37 d	43 d	51 bcd	65 bc	92 a
P. palmeri (1)	91 a	89 a	98 a	96 a	100 a	97 a	91 a
(2)	79 b	25 d	52 c	77 b	79 b	84 b	98 a
(3)	72 cd	61 d	83 bc	88 ab	95 ab	90 a	96 a
P. rostriflorus	45 b	49 b	79 a	87 a	97 a	94 a	92 a
P. strictus	88 a	93 a	99 a	90 a	93 a	97 a	93 a
GROUP II							
P. leiophyllus	6 e	8 e	25 d	28 cd	42 c	62 b	100 a
P. subglaber (1)	3 c	8 c	24 b	5 c	33 b	64 a	75 a
(2)	5 c	6 bc	6 bc	2 c	3 c	14 b	91 a
(3)	12 cd	3 d	14 bc	6 cd	5 cd	28 b	96 a
GROUP III							
P. leonardii (1)	0 c	0 c	1 c	36 b	93 a	93 a	31 b
(2)	0 c	0 c	0 c	8 bc	70 a	66 a	13 b
P. platyphyllus	1 d	21 c	55 b	66 b	99 a	97 a	53 b
P. sepalulus (1)	2 e	8 de	20 cd	29 bc	83 a	85 a	49 b
(2)	0 d	5 d	21 c	48 b	93 a	88 a	28 bc
(3)	0 c	6 c	35 b	35 b	73 a	85 a	42 b
GROUP IV							
P. cyananthus (1)	0 c	4 c	1 c	0 c	25 b	62 a	25 b
(2)	1 d	1 d	7 cd	15 c	60 b	88 a	44 b
(3)	5 cd	0 d	6 c	10 c	56 a	71 a	56 a
P. eatonii (1)	0 c	0 c	0 c	0 c	4 c	74 a	37 b
(2)	2 ef	1 f	5 de	10 d	35 c	84 a	60 b
P. fremontii	0 c	3 c	4 c	1 c	31 b	72 a	19 b
P. humilis (1)	0 d	0 d	0 d	4 d	48 c	84 a	62 bc
(2)	4 d	4 d	25 c	65 ab	86 a	77 a	49 b
P. laevis	8 c	9 c	26 b	80 a	86 a	99 a	88 a
P. whippleanus	0 c	2 bc	1 bc	1 bc	1 bc	6 b	42 a

[z]Within a collection, means followed by the same letter are not significantly different at the $p < 0.05$ level (SNK).

Table 3. Germination percentages[z] of 16 species of penstemon in response to four GA_3 concentrations.

Collection	Concentration of GA_3 (ppm) 0	50	150	250	500
GROUP I					
P. ambiguus	47 c	83 b	100 a	100 a	97 a
P. pachyphyllus	71 b	92 a	100 a	91 a	90 a
P. palmeri (3)	72 b	94 a	100 a	96 a	95 a
P. rostriflorus	45 c	83 b	97 a	92 a	91 a
P. strictus	88 a	87 a	100 a	93 a	98 a
GROUP II					
P. leiophyllus	6 c	80 b	98 a	100 a	100 a
P. subglaber (3)	12 c	73 b	99 a	96 a	97 a
GROUP III					
P. leonardii (2)	0 b	9 a	5 ab	13 a	13 a
P. platyphyllus	1 b	45 a	40 a	53 a	37 a
P. sepalulus (3)	0 b	20 a	22 a	42 a	42 a
GROUP IV					
P. cyananthus (3)	0 d	23 c	61 b	56 b	85 a
P. eatonii (2)	2 b	—	—	60 a	—
P. fremontii	0 b	—	—	19 a	—
P. humilis (2)	4 c	29 b	53 ab	56 ab	72 a
P. laevis	8 d	47 c	79 ab	88 a	74 b
P. whippleanus	0 c	12 b	41 a	42 a	49 a
Means	19.2	53.7	69.7	70.4	73.3

[z]Within a collection, means followed by the same letter are not significantly different at the $p < 0.05$ level (SNK).

one *P. palmeri* collection were also negatively affected by chilling. Clearly, stratification will not solve all penstemon seed dormancy problems. Group I was also characterized by complete germination at the lower concentrations of GA_3. The minimum concentration for complete germination was 50 ppm for *P. palmeri* and *P. pachyphyllus* and 150 ppm for *P. rostriflorus* and *P. ambiguus* (Table 3).

Group II. As might be expected from their high-elevation habitats, these four collections consisting of two species, *P. leiophyllus* and *P. subglaber*, had long chilling requirements. None of these collections had complete germination after 16 weeks of stratification and two of the *P. subglaber* collections were below 30% (Table 2). However, GA_3 treatments of 150, 250, and 500 ppm resulted in complete germination for these collections (Table 3). Thus, by one measure (response to stratification) these collections were among the most dormant tested, while by another measure (percent germination in GA_3) they were among the least dormant.

Group III. The three species (*P. leonardii, P. platyphyllus*, and *P. sepalulus*) of this group are taxonomically close and occupy similar mid-evaluation habitats. Consequently, their similarity in germination response is not surprising. *P. platyphyllus* was the only species in the experiment that responded favorably to 2 weeks of stratification. The collections of *P. leonardii and P. sepalulus* required 4 to 8 weeks for a similar germination response. Mean germination at 12 weeks was 85% and was not significantly different than that at 16 weeks for any of the six collections (Table 2). Germination for 50 ppm GA_3 was below 50% for all collections in this group (Table 3). Increasing the concentration to 150, 250, and 500 mg/l had no effect on germination. In general, these species responded well to intermediate periods of stratification and poorly to all concentrations of GA_3.

Group IV. The remaining six species (*P. cyananthus, P. eatonii, P. fremontii, P. humilis, P. laevis*, and *P. whippleanus*) are included in Group IV. This group does not have the taxonomic closeness of Group III. In addition, the 10 collections are representative of greater variability in habitat. These facts account for the heterogeneity of response found within this group. For the most part, the collections in this group had longer chilling requirements than those of Group III (Table 2). The high-elevation *P. whippleanus* collection failed to respond to any stratification treatment. Similarly, the high-elevation collection of *P. eatonii* did not respond to stratification treatments of less than 16 weeks. The *P. fremontii, P. cyanathus, P. eatonii* (2), and *P. humilis* (1) collections showed essentially no response to stratification of less than 12 weeks. Mean germination for these collections was 43% at 12 weeks and 77% at 16 weeks. In contrast to the rest of this group, the *P. humilis* (2) and *P. laevis* collections responded reasonably well to intermediate stratification (4 and 8 weeks). Both of these collections were taken from a site with relatively short mild winters, evidence of the importance of habitat at the site of seed origin.

Germination response to the higher concentrations of GA_3 was better in this group than in Group III. However, unlike that group, 50 ppm was never adequate for maximum response (Table 3).

GA_3 concentration. The uniformity of response from 150, 250, and 500 ppm GA_3 is in agreement with the results of another study on penstemon germination (Kitchen and Meyer, unpublished data) in which there were no significant differences using 250, 500, 750, and 1,000 ppm. This suggests that the inability of GA_3 to break dormancy in penstemon seeds is not simply a problem of permeability in the seedcoat or other embryonic coverings.

Stratification and GA_3. There was no significant correlation between dormancy as measured by response to GA_3 and dormancy as measured by response to stratification. Apparently germination response to GA_3 cannot be used as a predictor of stratification requirement for penstemon seeds.

Stratification and GA_3 did interact synergistically when applied in combined treatments, however. Chilling in a solution of GA_3 (250 ppm) significantly reduced the stratification requirement for species from Groups III and IV. The amount of stratification needed to reach 75% germination was shortened by 4 weeks for the *P. fremontii* collection and by 8 weeks for the *p. leonardii, P. humilis*, and *P. eatonii* collections (Fig. 1). The *P. sepalulus* and *P. platyphyllus* collections required 10 and the *P. cyananthus* collection 12 fewer weeks. The *P. whippleanus* collection failed to respond significantly to even 16 weeks of stratification without GA_3; however, when GA_3 was included with 8 weeks of prechill, germination reached 84%. Enhancement of germination by combining stratification and GA treatments has been demonstrated previously on a variety of landscape plants (1) and native perennials (14).

There was a tendency for seeds to germinate during prolonged stratification. The germination of seeds in the cold frequently began well before other seeds in the same petri dish had been rendered nondormant (Fig. 1). This variability in dormancy among the seeds from a single population has obvious adaptive value in unpredictable environments; however, uniformity in germination is preferred for horticultural uses. This problem was reduced when GA_3 was used with shorter chilling treatments.

These results verify that the seeds of many Intermountain species of penstemon, when collected from native populations, require extensive treatments to break dormancy. Though stratification for moderate periods (8 to 12 weeks) was effective for several species, it often resulted in uneven germination. This problem occurs because dormancy within a single population of seeds can be quite variable and because, like many species, most penstemon seeds are capable of germinating at temperatures near freezing. Penstemon propagation from seed would require either planting before stratification or transplanting as germinants. Several collections from higher elevations (Groups II and IV) responded poorly to short and moderate stratification. This fact coupled with the problems of uneven germination in the cold makes seed propagation of these species especially difficult.

On the other hand this study revealed considerable variability in seed dormancy among collections of the same species. The five species in Group I were sufficiently nondormant to be propagated from seed without any special treatment though caution is warranted as more dormant lots of each are likely to exist (2). Widely adapted species may exhibit a wide range in seed dormancy. With such species, careful selection from populations with low seed dormancy could reduce or eliminate much of the trouble associated with seed dormancy.

Other approaches might be used to modify penstemon seed dormancy. Environmental conditions during ripening (i.e. temperature and photoperiod) are known to affect seed dormancy (7, 11). Controlling these conditions may be one way of reducing seed dormancy in penstemons. Also, the seeds of many species gradually lose their dormancy in dry storage. This afterripening process occurs with at least some penstemons (2, 17). The use of aged seed in propagation may be another method for reducing the problems associated with penstemon seed dormancy.

Dormancy problems of some penstemon species are minimized when GA_3 is used. Stratification requirements are greatly reduced or eliminated. Germination does not occur during these shorter chilling periods thus enhancing uniformity and permitting post-treatment sowing. Unfortunately, applying GA_3 at concentrations effective for breaking seed dormancy may promote undesirable seedling development such as excessive shoot elongation. The effect of GA_3 on penstemon seedling quality is yet undetermined.

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