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Germination of *Phlox pilosa* L. Seeds Is Improved by Gibberellic Acid and Light but Not Stratification, Potassium Nitrate, or Surface Disinfestation

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Abstract. **The effects of warm stratification and cold stratification, gibberellin-3 (GA_3) concentration, potassium nitrate concentration, light, and duration of surface sterilization on the germination of downy phlox *(Phlox pilosa L.)* seeds were studied. Germination after 21 days (G21), days to 50% germination (T_{50}), and number of days between 10% and 90% final germination (T_{90}—T_{10}) were calculated for each treatment. Total germination percentage was most significantly improved by cold stratification at 5 2 °C for 10 weeks after warm stratification at 20 °C for 2 weeks; however, a substantial amount of germination occurred during the prestratification period, thus resulting in a crop with poor uniformity. A total of 10 mg•L^{-1} GA_3 significantly improved the G21, T5o, and T_{90}—T_{10} values. Although GA_3 concentration and duration of cold stratification period interacted significantly when the two were combined, the additive effects of GA_3 and cold stratification did not significantly improve G21 values over those obtained with GA_3 alone nor were T_{50} values improved over those obtained with cold stratification alone. Potassium nitrate did not influence the T_{50} and T_{90}—T_{10} values and improved G21 only slightly. Light was found to be necessary for germination. Surface sterilization with 10% bleach decreased the growth of fungi on seeds but had no significant effect on the germination responses of P. *pilosa* seeds. Application of GA_3 at 10 mg-L^{-1} is a promising method for improving seed germination in perennial *Phlox* species.**

The genus *Phlox* consists of ~ 60 species native to North America (Steffey, 1987; Wherry, 1955). The *"Phlox pilosa* complex," a taxonomic grouping considered to be a natural phyletic unit, consists of four species: *P. pilosa, P. divaricata, P. amoena,* and *P. floridana* (Levin, 1966; Wherry, 1955). The perennial species *Phlox pilosa* (also known as Prairie Phlox or Downy Phlox) consists of several subspecies (Levin, 1966). All species in the *"Phlox pilosa* complex" are self-incompatible, but there is a great deal of compatibility and potential for outcrossing within the complex (Levin, 1966).

Wildflowers are now widely used in residential and commercial landscaping, habitat restoration, and highway beautification projects (Milstein, 2005). *Phlox pilosa is* potentially useful in these situations. This species is found in all states from North Dakota to Texas and eastward into Florida and New York (Barkley, 1986). Germination of P. *pilosa* seeds is erratic (Specialty Perennials, 2006), and poor seedling emergence in its natural habitat has been reported (Christiansen, 1967). The inconsistent germination of many perennial species has long discouraged growers from starting plants from seed; however, several companies have recently begun to focus on improving germination of perennial species through seed treatments (Hamrick, 2005).

Although considerable research has been done on seed germination of the annual species P. *drummondii* (Carpenter et al., 1993a, 1993b, 1995), there is a paucity of scientific literature concerning germination in the perennial *Phlox* species. Several seed companies have published recommendations for germinating perennial *Phlox* seeds. For example, Prairie Moon Nursery (2004) suggests cold stratification at 33 to 38 OF (0 to 5 °C) for 2 months to break dormancy in P. *pilosa* seeds. Specialty Perennials (2006) recommends freezing P. *pilosa* seed for 2 weeks before sowing, then incubating seed trays in darkness at 70°F (20 °C) for 3 weeks, followed by 35 IF (2 °C) for 3 weeks before moving trays back to 70 IF (20 °C). Similarly, Jelitto Staudensamen (2004) recommends prestratification of P. *pilosa* seeds at 18 to 22°C in moist medium for 2 to 4 weeks, followed by cold stratification at —4 to 4 °C for 4 to 6 weeks, and a period (duration not specified) of moderate temperatures (5 to 12 °C) before exposure to warmer temperatures. Simplification of these procedures would be beneficial to commercial growers and seed producers. The Association of Official Seed Analysts (AOSA) makes recommendations for germinating *P. drummondii* but no other *Phlox* species (AOSA,1988).

Germination rate and uniformity of many wildflower species can be improved by cold stratification (Baskin and Baskin, 1998; Bratcher et al., 1993; Wartidiningsih et al., 1994). In their germination study of 91 Wisconsin prairie species, Greene and Curtis (1950) observed that germination of *Phlox pilosa* seeds increased from 2% to 10% after stratification at 40 IF (6 °C) for 3 months. The majority of prairie species tested by these researchers responded positively to cold stratification.

Release from dormancy by cold stratification is indicative of physiological dormancy, a condition in which the embryo lacks the ability to penetrate the seedcoat (Baskin and Baskin, 2005). Physiological dormancy is found in numerous plant families. Baskin and Baskin (2005) classify seeds in this category as having deep, intermediate, or nondeep physiological dormancy, depending on the temperature requirements for dormancy break, response to gibberellins, and the growth of embryos excised from seeds. The germination of some species with physiological dormancy is enhanced by a period of warm stratification (temperature not specified) before cold stratification (Baskin and Baskin, 2005).

In some species, cold stratification can replace a light requirement for germination (Bewley and Black, 1994). Recent studies have revealed that cold stratification has a direct effect on production of gibberellins (GAs) in seeds of *Arabidopsis thaliana* (Yamaguchi and Kamiya, 2000, 2002; Yamauchi et al., 2004). Exogenously applied GA_3 overcomes seed dormancy in several species (Baskin and Baskin, 1998; Hartmann et al., 1997) and promotes germination in some species that normally require cold stratification, light, or after-ripening (Bewley and Black, 1994). GA promotes the production of enzymes such as endo-β-mannanase, which loosen cell walls in the endosperm, thereby reducing resistance to radicle emergence (Bewley, 1997; Groot and Karssen, 1987: Yamaguchi and Kamiya, 2002).

In addition to its role in plant nutrition, nitrate acts as a signal molecule in several processes in plant development and metabolism (Wang et al., 2003). A solution of 0.2% potassium nitrate has been found to enhance germination of *Phlox drummondii* (Heft, 1957); however, Springer and Tyrl (1989) observed no significant enhancement of germination in seeds of P. *oklahomensis* treated with 0.2% KNO_3. Exogenously applied nitrate enhanced germination in *Arabidopsis* (Alboresi et al., 2005; Hilhorst and Karssen, 1988). Batak et al. (2002) found that exogenbusly applied nitrate reduced the light requirement for germination in seeds of *Arabidopsis* Landsberg erecta ecotype. The degree of dormancy in *Arabidopsis* seeds is correlated with nitrate nutrition of the mother

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plant and with the nitrate content of seeds themselves; the greater the available nitrate, the lower the dormancy (Alboresi et al., 2005). Nitrate accumulation in *Arabidopsis* seed is also correlated with a decreased requirement for GAs during germination (Alboresi et al., 2005).

Pathogenic fungi, bacteria, and viruses may be found on seedcoats or within seed tissues. Seedborne fungi may cause poor germination and impair seedling development (Halmer, 2000). Certain species *of Alternaria* can infect seed while it is still on the mother plant and can be responsible for decreased germination (Mycock and Berjak, 1995).

The present study was conducted to determine the effects of cold stratification with prestratification, GA_3, potassium nitrate, light, and surface disinfestation on the germination of *Phlox pilosa* seeds.

Materials and Methods

Germination methods. Phlox pilosa seeds were obtained from Prairie Moon Nursery (Winona, MN). Seeds were sown in 9-cm glass petri dishes on top of a single layer of blue blotter paper (Anchor Paper Company, St. Paul, MN). Except for Expts. 5 and 6, all seeds were treated one day after sowing with 3a,4,7,7a-tetrahydro-2-[(trichloromethyl) thio]-1H-isoindole-1,3(2H)-dione (captan), 50% wettable powder, formulated product at 0.24 mg/100 mL deionized water. Seeds in Expt. 5 were treated with captan on the day of sowing. Seeds in Expt. 6 were not treated with captan. Germination tests were performed in a growth chamber (model I-35LVL; Percival Scientific, Boone, IA) at 20 °C constant temperature with cool-white fluorescent lamps providing 50 ± 8 μ mol•m^{-2}•s^{-1} photosynthetic photon flux *(PPF).* The photoperiod was 12 h per 24-h cycle in all experiments. Placement of petri dishes in the incubator was completely randomized. Blotter paper was moistened as needed with deionized water.

Data collection. Four 50-seed replicates were used for each treatment unless stated otherwise. In all experiments except Expt. 5, germinated seeds (radicle 1 mm or greater) were counted daily for 21 d. Germinated seeds were discarded each day. In Expt. 5, 12 petri plates were kept in complete darkness. Four plates were selected at random and germinated seeds were counted at 7, 14, or 21 d after sowing. Seeds were discarded after counting. Concurrently, a control group of four petri plates was exposed to 12 h of irradiance per day, and germinated seeds were counted at 7, 14, and 21 d after sowing.

The final germination percentage at 21 d (G21), number of days to 50% final germination (T_{50}), and number of days between 10% and 90% germination (T_9 .T_{10}) were calculated for each treatment. Six experiments were conducted. All were performed as completely randomized designs.

Expt. 1: Effects of prestratification and cold stratification duration on germination responses. Seeds were sown in petri dishes on blotter paper moistened with deionized water and were prestratified (constant 20 °C and 12 h of irradiance per day) in the growth chamber for 0, 2, or 4 weeks. After prestratification, seeds were cold-stratified at 5 ± 2 °C in a dark refrigerator (Precision 812; Precision Scientific, Chicago) for 0, 2.5, 5, 7.5, or 10 weeks and were then returned to the 20 °C growth chamber for a poststratification period of 21 d.

Expt. 2: Effects of gibberellic acid on germination responses. Seeds were sown in petri dishes containing blotter paper moistened with GA_3 (grade III; Sigma-Aldrich Corp., St. Louis) at 0, 1, 10, 100, or 250 mg•L^{-1}in deionized water.

Expt. 3: Effects of potassium nitrate on germination responses. Seeds were sown in petri dishes on blotter paper moistened with KNO_3 (Sigma-Aldrich Corp.) at 0, 1, 5, 10, 50, or 100 mM in deionized water.

Expt. 4: Additive effects of GA_3 and cold stratification on germination responses. Seeds received one of the following treatments: 1) stratification for 10 weeks at 5 ± 2 °C in the dark; 2) plus GA3 (10 mg•L^{-1} in deionized water) applied to seeds on the day of sowing; 3) cold stratification for 10 weeks at 5 ± 2 °C plus GA3 (10 mg•L^{-1} in deionized water) applied to seeds on the day of transfer to germination conditions; or 4) untreated seed (control).

Expt. 5: Effects of light and darkness on germination responses. Seeds were sown on blotter paper moistened with deionized water. Petri dishes were sealed with parafilm after sowing. Seeds were incubated at constant 20 °C. There were two treatments: 1) exposure to light from cool-white fluorescent lamps (50 ± 8 μmol•m^{-2}•s^{-1} *PPF)* for 12 h daily; and 2) total darkness. petri dishes were wrapped in aluminum foil to exclude light.

Expt. 6: Seed disinfestation and pathology. Four groups of 20 seeds were used in each treatment. Seeds were treated with bleach (diluted with deionized water to 0.6% NaHC1O) for 10 or 20 min. Seeds were rinsed with sterile water and aseptically sown on acidified potato dextrose agar. Sterile conditions were maintained by sowing seeds under a laminar flow hood (Edgegard, Baker Co., Sanford, ME). Control seeds were rinsed with sterile water. Petri dishes were incubated for 3 don a laboratory bench at ambient temperature (~ 18 °C). Seeds were observed 3 d after sowing for the presence of fungi. Two additional replicates of 25 seeds per treatment were used for germination studies. Glass petri dishes and blotter paper were sterilized in an autoclave (Market Forge Sterilmatic, Everett, MA) at 121 °C and 103 kPa for 15 min. Seeds were sown under a laminar flow hood and petri dishes were transferred to the growth chamber for 21-d incubation. The experiment was repeated once.

Data analysis. The General Linear Models, analysis of variance, and t test procedures of SAS (SAS Institute, Cary, NC) were used for data analysis. Coefficients for orthogonal polynomial comparisons were calculated using the IML procedure of SAS. Data from Expt. 5 were transformed (arcsin for G21 and square root for T_{50} and T_{90}–T_{10}) before analyses.

Results and Discussion

Expt. 1: Effects of prestratification and cold stratification. A significant interaction occurred between prestratification and cold stratification periods as shown in Figure 1. The prestratification period had a more pronounced effect on G21 when seeds were

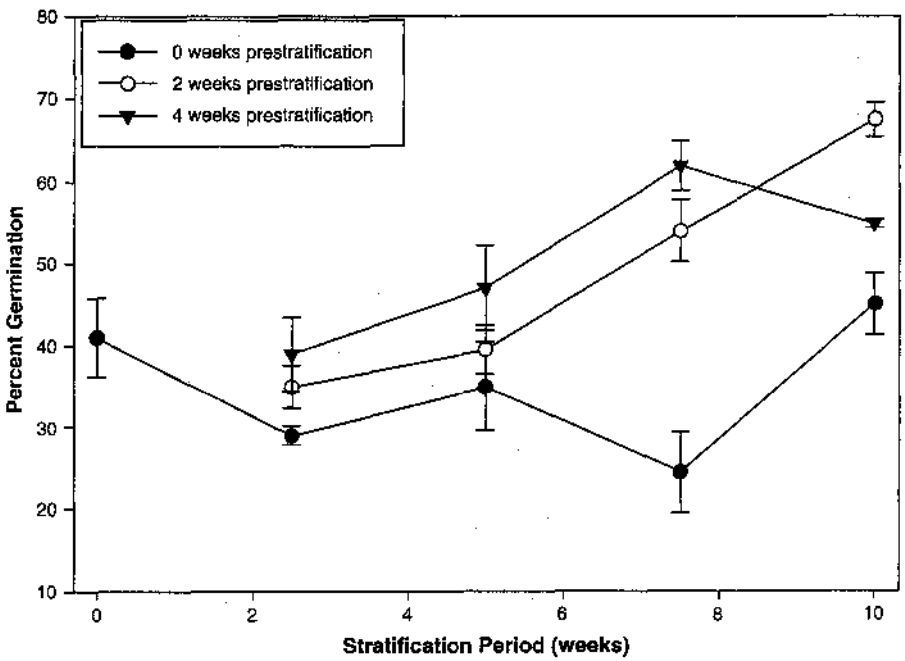

Fig. 1. Total germination percentage (G21) of cold-stratified *Phlox pilosa* seeds that received 0, 2, or 4 weeks prestratification at 20 °C. Standard error is represented by vertical bars.

subsequently cold-stratified for longer periods. With 2 weeks prestratification, G21 increased linearly as the duration of cold stratification increased. With 4 weeks prestratification, G21 increased as the cold stratification period increased to 7.5 weeks but declined slightly when seeds were stratified for 10 weeks.

Prestratification at 20 °C for 2 to 4 weeks induced germination in Q30% to 50% of the treated seeds (Table 1). An average of 34.5% of prestratified seeds germinated after 2 weeks at 20 °C, whereas an average of 43.5% germinated after 4 weeks. An analysis of covariance (data not shown) revealed that increasing the prestratification period decreased the ameliorative effect of cold stratification on G21. Significance in the 0-week prestratification category is artifactual because this group received no prestratification. Figures are presented in the interest of consistency.

The greatest total germination (67.5%) was achieved with 2 weeks prestratification and 10 weeks of cold stratification (Table 1). Although this is a significant improvement over the control, prestratification followed by cold stratification may not be a practical method for greenhouse growers who will be interested in producing a crop of plants that are uniform in size. Seedlings obtained during prestratification will be larger than those obtained after 10 weeks of stratification.

Greene and Curtis (1950) observed that germination *of Phlox pilosa* seeds increased from 2% to 10% after stratification at 40°F (6 °C) for 12 weeks. In the current study, G21 of seeds cold-stratified for 10 weeks without prestratification was 45%, slightly higher than the G21 value for the control (0 weeks prestratification, . 0 weeks cold stratification [Fig. 1]). It is possible that a period of cold stratification greater than 3 months with no prestratification is necessary for improvement of germination rate and uniformity in this species. Cold temperatures will keep nondormant seeds conditionally dormant (i.e., unable to germinate solely because of unfavorable temperature) while breaking dormancy in seeds with physiological dormancy.

In some temperate species, dormancy is broken by a period of warm temperatures followed by cold stratification. This response is most often associated with morphophysiological dormancy (Fenner and Thompson, 2005); however, seeds with morphophysiological dormancy have underdeveloped embryos (Baskin and Baskin, 2005). Dissection of 200 *P. pilosa* seeds under a binocular stereoscope (Zeiss, Thornwood, NY) revealed fully developed embryos in 96% of the seeds (data not shown). Thus, it appears that the *P. pilosa* seeds used in this experiment vary in their level of physiological dormancy. Variation in depth of seed dormancy may be incited by environmental factors or positional effects while seeds are maturing on the mother plant. Length of day, temperature, light quality, age, and nutritional status of the parent plant, seed position in the fruit, and position of the fruit in the inflorescence have been reported to affect seed genninability (Gray and Thomas, 1982; Gutterman, 1982). Greene and Curtis (1950) observed a wide variation in germination between seed lots collected in different years. These investigators surmised that this variation involved the same factors that are affected by stratification, but do not discuss these factors further (Greene and Curtis, 1950).

Expt. 2: Effects ofgibberellic acid. Exogenously applied GA_3 had a significant effect on G21, T_{50}, and T_s–T_{lo} values. G21 values exhibited a generally positive quadratic response, whereas T_{50} and T_{90}–T_{10} values showed positive and negative linear trends, respectively, to increasing GA_3 concentrations (Table 2). Application of 10 mg•L^{-1} GA_3 increased the G21 percentage to 68.5%, a significant improvement above the control. The T_{50} at 10 mg•L^{-1} was similar to that of the control, whereas T_{50} increased markedly at GA_3 concentrations greater than 10 mg•L^{-1}. A total of 10 mg•L^{-1} GA3 also shortened T_{90}–T_{10} to 4.5 d, 3 d less than the control.

Gibberellins have been reported to stimulate germination in seeds with nondeep and intermediate physiological dormancy (Baskin and Baskin, 1998). The results of this experiment demonstrate that GA_3 at 10 mg•L^{-1} shows promise as a practical method of improving the germination percentage, rate, and uniformity of P. *pilosa.*

Expt. 3.: Effects of potassium nitrate. Overall, application of KNO_3 had a significant negative effect on the G21 values (Table 3). These results are similar to those observed by Springer and Tyrl (1989) in their experiments with *P. oklahomensis. T*$_{50}$ and T90–T10 values were unaffected significantly by the KNO3 concentrations used in this study.

Nitrate acts as a signal molecule promoting germination in seeds of *Arabidopsis,* possibly through interaction with GA or abscisic acid production pathways (Alboresi et al., 2005). Although 5 mM KNO_3 improved germination of P. *pilosa* seeds above that of the control, the effect was marginal. Additional experiments are needed to test the effects of KNO3 concentrations between 1 and 10 mM.

Expt. 4: Additive effects of gibberellin-3 and cold stratification. Stratification and GA_3 treatments interacted significantly to affect G21, T_{50} , and T_{90}-T_{10} values. GA_3 without stratification markedly enhanced G21 to 55.5%, well above the control and other treatments, as shown in Figure 2A; however, the addition of 10 mg•L^{-1} GA_3 to stratified seeds did not significantly alter G21. Stratification without GA_3 had the most significant effect on the T_{50} value, reducing it to 4.75 d, as shown in Figure 2B. Addition of GA_3 to stratified seeds increased the T_{50} value to 7 d, a significant increase relative to stratification alone. Although the interaction between stratification and GA_3 was significant for the T_{90} $_{10}$ value, the effects of GA_3 on stratified and unstratified seeds were nonsignificant.

Expt. 5: Germination in light and darkness. Daily exposure of P. *pilosa* seeds to 50 ± 8 μmol•m^{-2}•s^{-1} *PPF* from cool-white fluorescent lamps yielded germination

Table 1. Effects of prestratification and stratification on germination of *Phlox pilosa* seeds.

Stratification period (weeks)	Percent germination prestratification	Percent germination poststratification	Total percent germination
	0 weeks prestratification		
2.5	0.0	29.0	29.0
5	0.0	35.0	35.0
7.5	0.0	24.5	24.5
10	0.0	45.0	45.0
Significance			
Linear	NS	**	*
Quadratic	NS	*	NS
Cubic	NS	***	**
Mean	0.0	33.4	33.4
	2 weeks prestratification		
2.5	30.5	4.5	35.0
5	28.0	11.5	39.5
7.5	38.0	16.0	54.0
10	41.5	26.0	67.5
Significance			
Linear	**	***	***
Quadratic	NS	NS	NS
Cubic	NS	NS	NS
Mean	34.5	14.5	49.0
	4 weeks prestratification		
2.5	37.0	2.0	39.0
5	43.0	4.0	47.0
7.5	50.5	11.5	62.0
10	43.5	11.5	55.0
Significance			
Linear	NS	*	***
Quadratic	NS	NS	*
Cubic	NS	NS	NS
Mean	43.5	7.3	50.8

NS, *, **, ***Nonsignificant or significant at $P = 0.05$, 0.01, or 0.001, respectively.

Table 2. Final germination percentage at 21 d (G21), T_{50}, and T_{90}–T_{10} values for *Phlox pilosa* seeds treated with different concentrations of GA_3.

GA_3 (mg/L)	Germination (%)	T_{50} (days)[z]	T_{90}-T_{10} (days)[y]
0	44.0	8.5	7.5
1	56.0	9.0	8.8
10	68.5	8.5	4.5
100	63.5	10.0	6.5
250	48.5	12.3	4.8
Significance	***	**	**
Linear	NS	***	**
Quadratic	***	NS	NS
Cubic	***	NS	***

[z]T_{50} = number of days until 50% of final germination.
[y]T_{90-10} = number of days between 10% and 90% of final germination.
[ns, **,***]Nonsignificant or significant at $P = 0.01$ or 0.001, respectively.

Table 3. Final germination percentage at 21 d (G21), T_{50}, and T_{90}–T_{10} values for *Phlox pilosa* seeds treated with different concentrations of KNO_3.

KNO_3 (mM)	Germination (%)	T_{50} (days)[z]	T_{90}–T_{10} (days)[y]
0	37.0	10.5	11.8
1	31.0	10.8	10.0
5	41.5	10.3	9.0
10	35.5	10.0	9.3
50	22.5	11.3	9.8
100	19.5	11.3	8.0
Significance	**	NS	NS
Linear	***	NS	NS
Quadratic	NS	NS	NS
Cubic	NS	NS	NS

[z]T_{50} = number of days until 50% of final germination.
[y]T_{90-10} = number of days between 10% and 90% of final germination.
[ns, **,***]Nonsignificant or significant at $P = 0.01$ or 0.001, respectively.

responses significantly greater than those seeds kept in total darkness (Table 4). Thus, we can conclude that light appears to be necessary for germination of this species at 20 °C. These, results differ from the observation of Carpenter et al. (1993b) that total germination percentage of P. *drummondii* seeds was unaffected by light or darkness. The seeds of most light-requiring species are physiologically dormant (Hartmann et al., 1997).

Expt. 6: Seed disinfestation and pathology. Disinfestation treatment had a significant effect on the presence of seedborne fungi (Table 5); however, it had no effect on the G21 or T_{50} values (data not shown). $T_{90—T10}$ was not calculated. The majority of fungi identified on the seeds were *Alternaria* and *Cladosporium* species. Rare colonies of *Fusarium* were observed. It appears that seedborne fungi are not the cause of poor germination in this species.

Conclusions

This study describes a set of experiments on the germination requirements of *Phlox pilosa* seeds. Ten weeks of cold stratification alone significantly decreased T_{50} in both Expts. 1 and 4, but did not improve G21. Total germination was improved to 67.5% after 2 weeks warm stratification and 10 weeks of cold stratification. This is a significant improvement over the control (41%); however, a substantial amount of germination during the warm stratification period means that this method would produce a crop

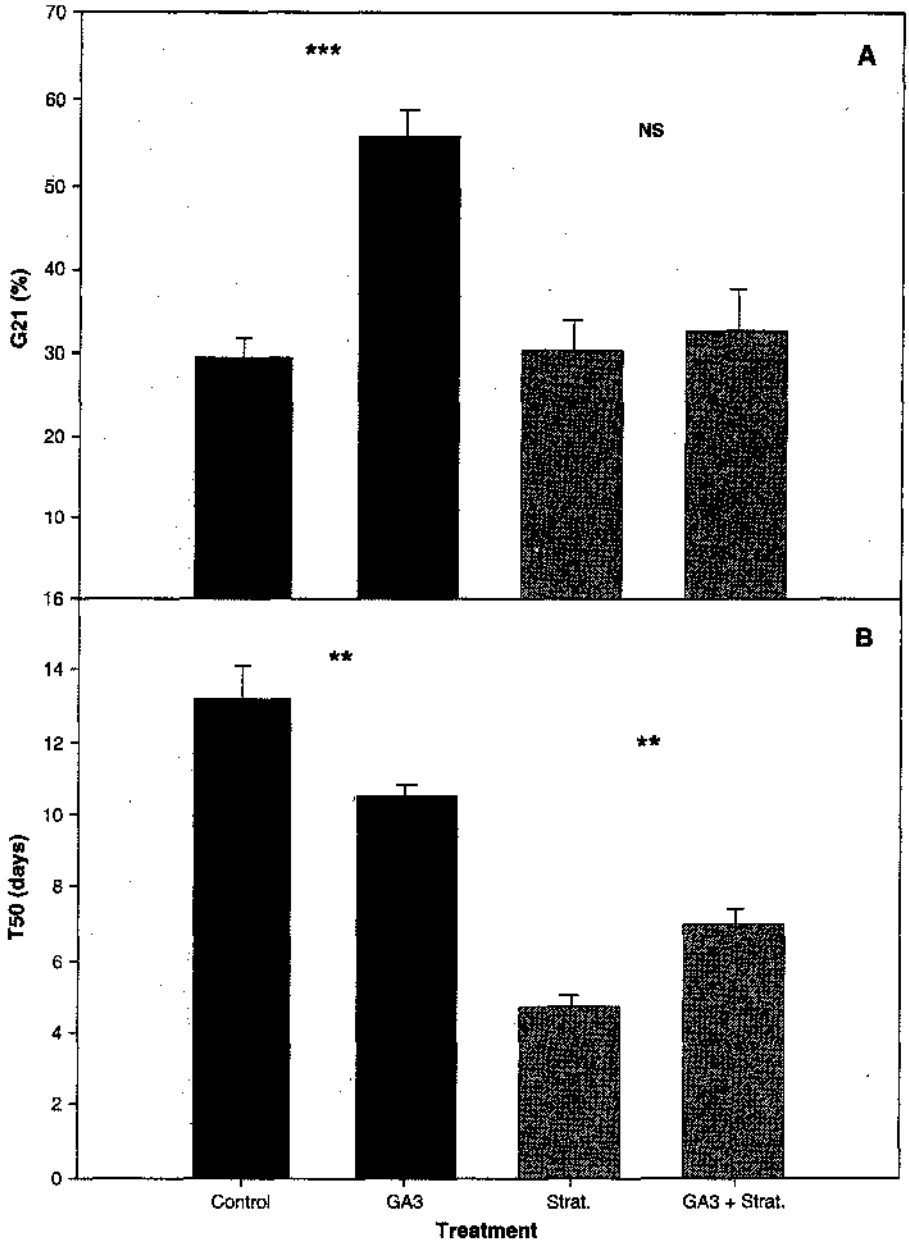

Fig. 2. Significance, as determined by F test, of the additive effects of GA_3 and stratification on G21 (A) and T_{50} (B) percentage of *Phlox pilosa* seeds. Strat = stratification. Standard error is represented by vertical bars. [ns, **,***]Nonsignificant or significant at $P = 0.01$ or 0.001, respectively.

Table 4. Germination responses of *Phlox pilosa* seeds to light and darkness.

Days after sowing	Germination (%) Dark[z]	Light[y]	Significance
7	0.5	14.0	***
14	1.5	37.5	**
21	2.5	44.5	**

[z]Petri plates wrapped in parafilm and aluminum foil on the day of sowing and checked for germination at 7, 14, and 21 d after sowing.
[y]Petri plates wrapped in parafilm on the day of sowing and exposed to 50 ± 8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux for 12 h daily. Germination was recorded at 7, 14, and 21 d after sowing.
[**,***]Significant at $P = 0.01$ or 0.001, respectively.

Table 5. Effect of surface disinfestation with 0.6% sodium hypochlorite on the presence of seedborne fungi in *Phlox pilosa*.

Duration of treatment (minutes)	Infected seeds (%)	Germination (%)	T_{50} (days)[z]
0	100	23.0	10.25
20	30	20.0	13.0
40	8.75	23.0	12.0
Significance			
Linear	***	NS	NS
Quadratic	***	NS	NS

[z]T_{50} = number of days until 50% of final germination.
[ns, ***]Nonsignificant or significant at $P = 0.001$.

with poor uniformity. Because one of the objectives in plant propagation is to attain a uniform crop, prestratification combined with poststratification would not produce desirable results. The procedures used in Expt. 1 are similar to the germination instructions for P. *pilosa* seeds published by Jelitto Staudensamen (2004). Prairie Moon Nursery (2004) suggests cold stratification for 8 weeks without prestratification. The results of the current study suggest that this treatment is insufficient for improving germination of P. *pilosa* seeds. It is possible that a period of more than 10 weeks of cold stratification is necessary.

Overall, the greatest improvements in both germination and uniformity were obtained by the addition of 10 mg•L^{-1} GA_3. This treatment improved germination to 68.5% and shortened T_{90}-T_{10} to 4.5 d. In Expt. 4, GA_3 significantly improved G21 over that obtained by 10 weeks of cold stratification, but did not improve G21 when added to stratified seeds. These results indicate that 10 mg•L^{-1} GA_3 may have a practical application for the improvement of germination percentage and uniformity in *Phlox pilosa*.

Application of KNO_3 at concentrations from 1 to 100 mM failed to bring about a substantial increase in germination. Surface sterilization with 10% bleach significantly decreased the incidence of seedbome fungi but did not influence germination, leading to the conclusion that seedbome fungi are not the cause of poor germination in *P. pilosa*.

Light is necessary for germination of this species at 20 °C. All of the germination experiments described were conducted at 20 °C, but there is no evidence that this is the optimum temperature for germination of this species. Except for the seeds kept in complete darkness in Expt. 4, all experiments used a 12-h photoperiod. Further experimentation is warranted to determine the germination responses of P. *pilosa* seeds at different temperatures and photoperiods (Specialty Perennials, 2006).

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