Temperature Requirements for the Storage and Germination of *Delphinium* × *cultorum* Seed

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Additional index words. cold pretreatment, seed desiccation, relative humidity

Abstract. *Delphinium* seed germination was about equal in light or darkness. Seed total germination percentages were highest and about equal at constant 15 or 20C and alternating (12 h) 10/20C, 15/25C, or 20/30C. The most rapid and uniform germination generally occurred at constant 20C. Storing seeds dry at 2C for 3 weeks before germination reduced the days to 50% of final germination (T_{50}) and between 10% and 90% germination ($T_{90} - T_{10}$) but did not increase total germination. The seeds had only limited desiccation tolerance, with 'Magic Fountains Lavender' having declining germination percentages at moisture contents below 7.0% and 'Magic Fountains Lilac' below 6.7%. Seeds tolerated storage at low, nonfreezing or subzero temperatures, but cultivar responses differed. 'Magic Fountains Lavender' had progressively lower germination percentages as storage temperatures declined from 5 to -20C, while 'Magic Fountains Lilac' germination increased. The relative humidity (RH) and temperature that delphinium seed received during long-term storage influenced germination. Germination after seed storage at 5C was higher, earlier, and more uniform than after 15 or 25C storage. The highest total germination percentages occurred following seed storage at 5C and 30% to 50% RH, the shortest T_{50} from 35% to 55% RH, and shortest spans ($T_{90} - T_{10}$) from 25% to 50% RH.

Recently introduced dwarf pacific types of *Delphinium* × *cultorum* that are compact and early flowering have been gaining acceptance as bedding plants. Commercial producers need rapid and uniform germination from direct seeding in propagation trays, but only limited research has been conducted on the germination of delphinium seed. Post (1942) found little germination above 13C, while the yearbook of agriculture (U.S. Dept. of Agriculture, 1961) reported 1.5 to 21C as best, and Holcomb and Beattie (1990) recommended 21C medium and 27C air temperatures. Ball (1985) recommended that dry seed be chilled at 2C for 2 weeks before sowing for improved germination. Recommendations for delphinium seed storage appear to be lacking. Our research objectives were to determine the need for light or darkness during germination, to compare temperatures during germination, and measure the effects of seed chilling, desiccation, and relative hu-

midity (RH) during seed storage.

Seed handling and germination procedures. Seeds of 'Magic Fountains Lavender' and 'Magic Fountains Lilac' used in all studies were harvested by Bodger Seeds, Lompoc, Calif., on 23 July 1990; air-dried for 10 days; then milled before shipment. Seeds were dusted with 3 α ,4,7,7-tetrahydro-2-

[(trichloromethyl)thio] -1- *H* -isindole-1,3 (2 *H*)-dione (captan) after receipt on 9 Aug. All treatments contained four 100-seed replicates, with replicates germinated in individual 9-cm petri dishes on double layers of Whatman No. 1 filter paper moistened with 6 ml of distilled water (DW). Seeds were germinated in incubators (Stults Scientific Engineering Corp., Springfield, Ill.) at constant or 12-h alternating temperatures. In each study, germination counts were made daily of seeds with radicle protrusion through the testa. Total germination percentages (G), days to 50% of final germination (T_{50}), and germination span in number of days between 10% and 90% germination ($T_{90} - T_{10}$) were calculated as described by Furutani et al. (1985).

Germination temperature. 'Magic Fountains Lavender' and 'Magic Fountains Lilac' seeds were stored dry at 2 or 25C for 3 weeks before germination in darkness at constant 15, 20, 25, or 30C. In a second study, seeds stored at 2C for 6 weeks were germinated in dark incubators at 12-h alternating temperatures of 10/20C, 15/25C, or 20/30C. The design for both studies was a randomized block with data tested by means of analysis of variance (ANOVA); Tukey's HSD multiple-comparison procedure was used to compare alternating temperatures.

Seed moisture content. Four replications of 100 seeds for each treatment were weighed, placed in 9-cm open petri dishes, and dehydrated for 6, 12, 24, 48, 72, or 96 h in 40C forced-draft ovens. Following dehydration, seeds of each replication were reweighed and immediately sealed in screw-capped 10-ml glass vials, 100 seeds per vial, and stored at 2C for 3 weeks. After storage, seeds were reweighed and germinated at constant 20C. Germination data were analyzed by means of ANOVA and polynomial regression analysis. When the seeds were received, four 100-seed lots of each cultivar

Table 1. Comparison of delphinium germination at constant temperatures using seed stored for 3 weeks at 2 or 25C.

Cultivar	Storage temp (°C)	Germination ^z			
		Temp (°C)	Percent ^y	T_{50} ^y (days)	$T_{90} - T_{10}$ ^y (days)
Magic Fountains Lavender	2	15	78 a	7.6 b	8.9 b
		20	75 a	6.1 c	7.7 c
		25	70 a	7.3 b	9.2 b
		30	37 b	10.8 a	14.8 a
	25	15	78 a	10.8 a	11.2 b
		20	82 a	7.9 b	9.6 c
		25	64 b	8.2 b	10.8 b
		30	32 c	10.8 a	14.8 a
Magic Fountains Lilac	2	15	83 a	7.9 b	6.4 b
		20	83 a	6.4 c	5.1 c
		25	72 b	7.5 bc	6.8 b
		30	11 c	12.0 a	11.3 a
	25	15	82 ab	10.5 b	8.3 b
		20	86 a	7.8 c	6.8 c
		25	75 b	9.4 b	8.2 b
		30	16 c	14.2 a	13.0 a

^zValues represent the means of 400 seeds.

^yMean separation within each column among germination temperatures by Tukey's studentized range test ($P < 0.05$).

Received for publication 6 Dec. 1991. Accepted for publication 13 Apr. 1992. Florida Agriculture Experiment Station Journal Series no. R-01948. We acknowledge the seed source and assistance of Dana Richards, Bodgers Seeds, Lompoc, Calif. This research was partially sponsored by a grant from the Bedding Plants Foundation, P.O. Box 27241, Lansing, MI 48909. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

Fig. 1. Relationship between seed moisture content during storage and the subsequent total germination percentage (G) of delphinium 'Magic Fountains Lavender' ($\diamond$ —) and 'Magic Fountains Lilac' ($\blacklozenge$). Comparisons were made using polynomial regression analyses at $P = 0.05$. 'Magic Fountains Lavender': $\hat{G} = -127.50 + 52.95 \times \text{moisture} - 3.31 \times \text{moisture}^2$, $R^2 = 0.94$. 'Magic Fountains Lilac': $\hat{G} = -83.60 + 43.31 \times \text{moisture} - 2.78 \times \text{moisture}^2$, $R^2 = 0.86$.

Table 2. Delphinium seed germination at 20C and under 12-h alternating temperatures following 6 weeks storage at 2C.

Cultivar	Temp ^a (°C)	Germination		
		Percent	T ₅₀ (days) ^b	T ₉₀ - T ₁₀ (days) ^c
Magic Fountains Lavender	20	82	6.4	6.3
	10-20	84	7.5	6.8
	15-25	85	7.3	7.1
	20-30	83	8.1	7.7
Tukey's HSD, 5%		NS	0.6	0.7
Magic Fountains Lilac	20	85	6.6	5.2
	10-20	89	7.8	5.8
	15-25	87	7.3	6.0
	20-30	85	8.0	7.9
Tukey's HSD, 5%		NS	0.7	0.8

^aExcept for 20C, temperature alternated every 12 h.

^bMeans to 50% of final germination.

^cDays from 10% to 90% germination.

were weighed, dehydrated at 105C for 48 h, and reweighed after cooling to determine the initial moisture content.

Storage temperature. Treatments consisting of four 100-seed replicates were placed in 15 × 2.5-cm petri dishes on wire screens supported by segments of tubing 1 cm above a chemical desiccant. Constant RH (22%) was maintained in the dishes sealed with parafilm after 50 ml of saturated potassium acetate was added to the bottom of each dish (Copeland, 1976). Refrigerated incubators were maintained at 2C for 2 weeks, then

seeds were placed in 10-ml glass vials, sealed with screw caps, immersed in controlled-temperature polyethylene glycol-water (v/v) baths (Guy and Carter, 1984), and held for 7 days at 5, 0, -5, -10, -15, or -20C. Bath temperatures were lowered 3C/h to final temperatures, then increased 4C/h to 10C at the end of 7 days. Following temperature treatment, seeds were germinated in darkness at 20C. The design was a randomized block with data analyzed by means of ANOVA and linear regression analysis.

Temperature and relative humidity inter-

actions during seed storage. Seeds of 'Magic Fountains Lavender' were stored at 11%, 34%, 52%, 75%, or 95% RH at 5, 15, or 25C for 3, 6, 9, or 12 months. Humidity treatments were achieved as described previously, except saturated lithium chloride, magnesium chloride, magnesium nitrate, sodium chloride, or potassium nitrate maintained 11%, 34%, 52%, 75%, or 95% RH, respectively. Incubators maintained constant temperatures during seed storage or germination. Four 100-seed replications were germinated at 20C in darkness immediately following storage at each duration and RH level, while another four replications were desiccated in force-draft ovens for 48 h at 105C to determine the moisture contents of stored seed. Analysis of variance and response surface regression procedures were used to analyze the data. Within each storage temperature, a randomized complete block design was used with a factorial treatment structure (4 × 5) between relative humidity and storage period.

Temperature influenced seed germination, with G generally higher at a constant 15 or 20C than at 25 or 30C (Table 1). Mean T₅₀ and T₉₀ - T₁₀ generally were shorter at 20C than at 15, 25, or 30C. Our results indicated that 20C promoted faster and more uniform germination of delphinium and that the seeds germinated over a broad temperature range, as reported by the U.S. Dept. of Agriculture (1961). No differences in G were found among seeds receiving alternating temperatures at 12-h intervals or at a constant 20C (Table 2). Shorter T₅₀ occurred at constant 20C than at alternating 10/20C, 15/25C, or 20/30C. The germination spans were similar for seeds at constant 20C or alternating 10/20C but longer at 15/25C or 20/30C (Table 2). Hartmann et al. (1990) reported fluctuating day/night temperatures increased seed germination, with optimal diurnal variations of 10C. Our results indicated that the germination of delphinium was not increased by 12-h alternating temperatures.

Storing seed dry for 3 weeks at 2 or 25C failed to change the G at 15, 20, 25, or 30C germination temperatures (Table 1). Seeds stored at 2C for 3 weeks generally had significantly reduced T₅₀ and T₉₀ - T₁₀ for both cultivars (Table 1). Although Ball (1985) recommended chilling delphinium seed before sowing, no indication was given as to the nature or extent of the benefit.

Seed had only limited desiccation tolerance. 'Magic Fountains Lavender' seeds had 9.2% moisture and 'Magic Fountains Lilac' 8.4% moisture when received. Reducing 'Magic Fountains Lavender' seed moisture below 7.0% or 'Magic Fountains Lilac' moisture below 6.7% significantly lowered total germination (Fig. 1). Bewley and Black (1982) suggested that embryo cells are damaged during severe seed dehydration, especially the tonoplast and plasmalemma, both selectively permeable membranes. The capacity of seeds to maintain physiological integrity during dry storage and regain a high level of metabolism upon rehydration is a determining factor in seed vigor or viability.

Table 3. Coefficient estimates, R^2 values, and figure designations for the second-degree models; estimated response = $b_0 + b_1P + b_2RH + b_3P \times RH + b_4P^2 + b_5RH^2$.

Response	Temp (°C)	Coefficient estimates						R^2	Figure
		b_0	b_1	b_2	b_3	b_4	b_5		
G	5	63.2	-3.4	1.7	0.04	0.009	-0.03	0.78	3A
	15	92.8	-7.7	0.9	0.001	0.37	-0.02	0.81	3B
	25	76.8	-4.5	0.8	0.09	0.03	-0.02	0.93	3C
T_{50}	5	9.1	0.26	-0.12	-0.11	0.003	0.001	0.56	3D
	15	8.9	0.51	-0.13	-0.04	0.004	0.001	0.47	3E
	25	15.0	-0.37	-0.37	0.03	0.003	0.006	0.87	3F
$T_{90} - T_{10}$	5	8.5	0.3	-0.08	-0.02	0.002	0.008	0.46	3G
	15	8.2	0.6	-0.09	-0.04	0.005	0.001	0.56	3H
	25	13.7	-0.26	-0.24	0.03	-0.004	0.005	0.72	3I

Fig. 2. Effect of seed storage at low, nonfreezing and subzero temperatures on the total germination percentage (G) of delphinium 'Magic Fountains Lavender' ($\diamond$ —) and 'Magic Fountains Lilac' ($\blacklozenge$ —). Data were analyzed by linear regression analysis ($P = 0.05$). 'Magic Fountains Lavender': $\hat{G} = 81.07 - 0.19 \times \text{temp}$, $R^2 = 0.24$. 'Magic Fountains Lilac': $\hat{G} = 88.09 - 0.14 \times \text{temp}$, $R^2 = 0.20$.

The seeds of both cultivars were tolerant of low, nonfreezing and subzero storage temperatures, but the cultivars responded differently to low temperatures during germination (Fig. 2). 'Magic Fountains Lavender' germination declined with storage temperatures between 5 to -20°C, while that of 'Magic Fountains Lilac' increased as temperatures declined. 'Magic Fountains Lavender' had higher seed moisture (7.8%) than 'Magic Fountains Lilac' (6.9%) during freezing, which possibly contributed to the reduced germination.

The RH and temperature during seed storage determined the total germination after storage. Highest total germination occurred after 5°C storage at 30% to 50% RH, although germination percentage declined at

long storage durations (Fig. 3A). Total germination percentages declined when RH was above or below the 30% to 50% range. Storing seeds at 15°C generally led to lower G than storage at 5°C (Fig. 3B). Highest total germination at 15°C was at 25% RH, although differences among treatments between 10% and 40% RH were small. Larger losses in seed viability at 15°C than at 5°C occurred above 60% RH. Seeds stored at 25°C had lower total germination after storage than those stored at 5 or 15°C at all relative humidity levels (Fig. 3C).

Seeds achieved T_{50} in fewer days after storage at 5°C than at 15 or 25°C (Fig. 3D-F). Germination T_{50} required fewest days after 5°C storage at 35% to 55% RH, with longer periods required at higher or lower RH levels

(Fig. 3D). At 15°C, shortest periods to T_{50} occurred after 25% to 50% RH seed storage, but differences in T_{50} among relative humidities were small, except above 70% (Fig. 3E). Storing seeds at 25°C delayed germination by lengthening T_{50} values, with longest delays for seeds stored above 50% RH (Fig. 3F).

The $T_{90} - T_{10}$ values generally were lengthened by storing seeds at high temperatures and high relative humidities (Fig. 3G-I). Although fewer days to $T_{90} - T_{10}$ were required after storage at 5°C than at 15°C, both had uniform germination spans below 40% RH (Fig. 3G and H). At 25°C, the relative humidity level during seed storage governed the length of $T_{90} - T_{10}$ (Fig. 3I). Relative humidity level during seed storage at 25°C had more effect on G, T_{50} , or $T_{90} - T_{10}$ than on extending the storage period from 3 to 12 months (Fig. 3A-I).

This study presents information for optimizing the environmental conditions during the storage and germination of delphinium seed. The seed had no requirement for light or darkness to terminate germination and had similar G and T_{50} (data not presented) for seed germinated at 20°C in darkness or in 24 h irradiation (60-W incandescent lamps at $75 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Highest G occurred at a constant 15 or 20°C, germination temperatures recommended in earlier reports. Similar G and frequently shorter T_{50} or $T_{90} - T_{10}$ were found among seeds germinated at a constant 20°C than at alternating (12 h) 10/20°C, 15/25°C, or 20/30°C. Carpenter and Ostmark (1988) and Carpenter et al. (1991) reported amaryllis (*Hippeastrum* $\times$ *hybridum* Hort) and gladiolus (*Gladiolus grandiflorus* Andr.) had maximum G at optimum constant temperatures and significantly lower levels at 12-h alternating temperatures.

Our research reaffirmed that storing delphinium seeds dry at 2°C promoted improved germination after storage (Ball, 1985). Storing seeds dry at 2°C, rather than 25°C, promoted both earlier T_{50} and shorter $T_{90} - T_{10}$. No differences were found between the G, T_{50} , or $T_{90} - T_{10}$ of seeds stored at 2 and 5°C for 5 weeks (data not presented). Delphinium seeds were tolerant of low, nonfreezing and subzero temperatures during storage at 6.9% moisture content. Barton (1932) reported that annual and perennial delphinium seed had no significant loss in G or vigor after storage for 5 years at -15°C.

Improved practices and technology in the seed production industry have promoted the

Fig. 3. Contour plots for total germination percentages (A-C), days to 50% of final germination (D-F), and days between 10% and 90% germination (G-I) for seeds germinated at 20C following storage at 11% to 95% RH for 3 to 12 months at 5C (A, D, G), 15C (B, E, H), or 25C (C, F, I). Regression equations used to generate these plots are listed in Table 3.

drying of freshly harvested seeds to very low moisture levels for storage and shipment in sealed packages. It is very important to know whether drying causes losses in seed viability or vigor. Our study showed that a significant decline in G occurred when delphinium seed moisture contents declined below 6.7% to 7.0%, which indicated the importance of controlling and limiting the moisture loss from the seed. Although the data are not presented, significant lengthening of T_{50} and $T_{90} - T_{10}$ also occurred as seed moisture levels declined below 6.7% to 7.0%. Justice and Bass (1978) reported that delayed and irregular germination are the first indications of seed injury from storage at excessively low moisture contents.

Seed moisture content during storage affects the rate of seed deterioration. In storage, the moisture content of seeds will come to equilibrium with the RH of the surrounding air. Delphinium seed stored 12 months at 5C and 11%, 34%, 52%, 75%, or 95% RH had 4.9%, 6.8%, 8.3%, 11.1%, and 15.4% moisture contents, respectively. Ac-

cording to computer-generated estimates, the highest G occurred following seed storage at 5C and 30% to 50% RH, which resulted in -6.6% to 8.2% seed moisture content. Shortest T_{50} s were after 5C storage at 35% to 55% RH, or 6.8% to 8.5% moisture contents, and shortest $T_{90} - T_{10}$ after 5C storage at 25% to 50% RH, or $\approx$ 6.1% to 8.2% seed moisture.

Literature Cited

- Ball, V. 1985. Ball red book: Greenhouse growing. 14th ed. Reston Publishing Co., Reston, Va.
- Barton, L.V. 1932. Effect of storage on the viability of delphinium seeds. Boyce Thompson Inst. Contrib. 4:141-153.
- Bewley, J.D. and M. Black. 1982. Physiology and biochemistry of seeds. Springer-Verlag, New York.
- Carpenter, W.J. and E.R. Ostmark. 1988. Sensitivity of seed germination of amaryllis to light and temperature. HortScience 23:1002-1004.
- Carpenter, W.J., G.J. Wilfret, and J.A. Cornell. 1991. Temperature and relative humidity govern germination and storage of gladiolus seed. HortScience 26:1054-1057.
- Copeland, L.O. 1976. Principles of seed science and technology. Burgess, Minneapolis.
- Furutani, S.C., B.H. Zandstra, and H.C. Price. 1985. Low temperature germination of celery seeds for fluid drilling. J. Amer. Soc. Hort. Sci. 110:149-153.
- Guy, C.L. and J.V. Carter. 1984. Characterization of partially purified glutathione reductase from cold hardened and nonhardened spinach leaf tissue. Cryobiology 21:454-464.
- Hartmann, H.T., D.E. Kester, and F.T. Davies, Jr. 1990. Plant propagation principles and practices. 5th ed. Prentice Hall, Englewood Cliffs, N.J.
- Holcomb, E.J. and D.J. Beattie. 1990. Potted delphiniums. Grower Talks 8:14-15.
- Justice, O.L. and L.N. Bass. 1978. Principles and practices of seed storage. U.S. Dept. Agr. Hdbk. 506, Washington, D.C.
- Post, K. 1942. Effects of daylength and temperature on growth and flowering of some florist crops. Cornell Agr. Expt. Sta. Bul. 787:1-70.
- U.S. Dept. of Agriculture. 1961. Seeds. The yearbook of agriculture. U.S. Dept. Agr., Washington, D.C.