Growth Regulators and Storage Temperature Govern Germination of *Coreopsis* Seed

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Abstract. The storage and germination environments were evaluated to determine the cause of low total germination percentages and highly irregular germination of *Coreopsis lanceolata* L. seed. Highest total germination and most rapid and uniform germination of seed occurred at constant 15C, other constant temperatures and all alternating temperature regimes caused lower total germination or delayed it. Seeds tolerated -20C during storage, but total germination was reduced below -5C. Recently harvested seeds had 44% total germination, but 54% to 81% germination was achieved after 6 hours of soaking seeds in 1000 ppm GA₃, 1000 ppm ethephon, or 25 ppm kinetin alone or in combination. Growth regulators reduced the number of days to 50% of final germination (T₅₀), and the span in days between 10% and 90% of germination (T₉₀ - T₁₀). Storing fresh seeds without chemical treatment for > 6 months at 5C and 10% to 20% relative humidity (RH), or 15C at 20% to 35% RH, increased total germination to 75% and 80%, respectively. Ten days were required to achieve T₅₀ after 5 to 6 months of storage at 5C and 10% to 20% RH or 15C and 10% to 40% RH, with longer periods to T₅₀ at other storage durations and RH levels. The germination spans (T₉₀ - T₁₀) were lengthened the higher the seed storage temperatures between 5 to 25C, with longer spans as seed storage durations and relative humidities increased. Total germination was similar after storing seeds at 5 or 15C and 10% to 30% RH and after soaking recently harvested seeds in GA₃ + ethephon, but the days to T₅₀ and T₉₀ - T₁₀ were shorter after growth regulator treatment. Chemical names used: (2-chloroethyl) phosphonic acid (ethephon); gibberellic acid (GA₃); 6-furfurylaminopurine (kinetin).

Wildflowers are gaining acceptance as bedding plants for low-maintenance urban landscapes. Direct seeding is the most economic method of propagation, but low total germination and nonuniform plant stands can result. Many companies and nurseries specialize in producing wildflower seeds and plants, but limited information is available on seed handling, storage, or germination. The optimal conditions for germinating wildflowers and the mechanisms controlling seed dormancies are unknown.

Kaspar and McWilliams (1982) reported *Coreopsis tinctoria* Nutt. seeds germinate at 15 to 35C, with the optimum at 30C. Ball (1985) recommended germination temperatures from 18 to 24C for *C. grandiflora* Hogg ex Sweet seeds. Samfield et al. (1988, 1990) found seed osmoconditioning (priming) in aerated solutions of 50 mM potassium phosphate buffer at 16C for 3 days increased *C. lanceolata* total germination by 9 percentage points (from 33% to 42%) and improved germination uniformity and earliness. The re-

search objectives of our experiments were to develop handling, storage, and germination recommendations for *C. lanceolata* seed and to determine if plant growth-regulating chemical treatment or proper storage promotes earlier, more uniform, increased total germination.

Germination temperature. Seeds of tickseed were collected from established natural stands in north-central Florida in late May 1990. Seeds were harvested from plants with mature-ripe, naturally dehiscent seed heads. Seeds in all studies were cleaned, then dusted

Table 1. Influence of temperature on the germination of *Coreopsis lanceolata* seed. Data are the means of 400 seeds.

Temp (°C)	Germination		
	G ^z	T ₅₀ ^y	T ₉₀ - T ₁₀ ^x
10	22	16	13
15	48	10	8
20	33	14	11
25	7	18	15
30	0	---	---
Significance			
Linear	*	NS	NS
Quadratic	**	**	**
Cubic	*	NS	NS

^zPercent total germination after 36 days.

^yDays to 50% final germination.

^xDays from 10% to 90% germination.

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

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Fig. 1. Total germination percentages of *Coreopsis lanceolata* seed in darkness after low-temperature seed storage for 7 days. Data are the means of 400 seeds.

Fig. 2. 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 15°C in darkness following storage at 11% to 75% relative humidity for 3 to 9 months at 5°C (A, D, G), 15°C (B, E, H), or 25°C (C, F, I). Regression equations used to generate these plots are listed in Table 4.

with 3 α ,4,7,7 α -tetrahydro-2-[(trichloromethyl)thio]-1H-isindole-1,3(2H)-dione (captan) before storage or germination. All experiments were conducted with four randomized complete blocks, with 100-seed replicates in each treatment. Seeds of each replicate were germinated in individual 9-cm petri dishes on a double layer of Whatman no. 1 filter paper moistened with 5 ml distilled water (DW). In a preliminary study, seeds were germinated in darkness or in continuous incandescent light at 20 μ mol-m⁻²-s⁻¹ of photosynthetically active radiation (PAR) in

incubators at constant 20°C. Similar total germination percentages were found in light or darkness. In the initial study, seeds stored 2 months at SC were germinated in dark incubators at constant 10, 15, 20, 25, or 30°C. In a subsequent study, comparisons of germination were made between constant 15°C and 12 h alternating temperatures of 5/15°C, 10/20°C, 15/25°C, or 20/30°C. In both studies, daily germination counts were made of seeds with visible radicle protrusion through the testa. Total germination percentages (G), days to 50% of final germination (T₅₀), and ger-

Table 2. Germination of *Coreopsis lanceolata* seed under alternating temperatures. Data are the means of 400 seeds.

Alternating ^z temp (°C)	Germination		
	G ^y	T ₅₀ ^x	T ₉₀ - T ₁₀ ^w
15	51	10	9
5/15	32	31	20
10/20	46	23	16
15/25	41	16	14
20/30	36	19	17
Tukey's HSD, 5%	5	7	6

^zTemperature alternated every 12 hours.

^yPercent total germination after 45 days.

^xDays to 50% final germination.

^wDays from 10% to 90% germination.

Table 3. *Coreopsis lanceolata* germination after soaking seed for 6 h in 1000 ppm ethephon, 1000 ppm gibberellic acid (GA₃), or 25 ppm kinetin alone or in combination as pregermination treatments. Data are the means for 400 seeds.

Treatment	Germination ^z		
	G ^y	T ₅₀ ^x	T ₉₀ - T ₁₀ ^w
Distilled water	44 c	11.2 a	12.1 a
Ethephon (E)	73 ab	7.1 bc	6.5 bc
GA	67 b	6.6 cd	5.7 c
Kinetin (K)	54 c	10.9 a	11.6 a
GA + K	66 b	7.5 b	7.0 b
GA + E	79 a	6.1 d	5.5 c
K + E	69 b	6.6 cd	5.8 c
GA + E + K	81 a	6.3 d	5.7 c

^zMean separation within columns by Tukey's HSD test, P = 0.05.

^yPercent total germination at 15°C after 21 days.

^xDays to 50% final germination.

^wDays from 10% to 90% germination.

mination span in number of days between 10% and 90% germination (T₉₀ - T₁₀) were calculated as recommended by Furutani et al. (1985) in these and subsequent studies. Data were analyzed using analysis of variance and multiple regression procedures.

Growth regulators. Immediately after harvest, seeds were soaked 6 h in 1000 ppm ethephon, 1000 ppm GA₃, or 25 ppm kinetin alone or in combinations. Nontreated seeds were soaked 6 h in DW. After treatment, all seeds were vacuum-dried at room temperature for 30 min, stored dry 2 days, then germinated at constant 15°C as previously described. Seeds with radicles were counted daily, and G, days to T₅₀, and T₉₀-T₁₀ calculated. Data were analyzed by Tukey's honestly significant difference test at P = 0.05.

Cold tolerance of seed during storage. Seeds were placed in 15 × 2.5-cm petri dishes on wire screens supported by segments of tubing 1 cm above a chemical desiccant. Constant 22% RH was maintained in the sealed petri dishes by adding 50 ml of saturated potassium acetate to the bottom of each dish (Copeland, 1976). A refrigerated incubator was maintained at 15°C during the week of seed dehydration. Following dehydration, seeds were placed immediately in 10-ml sealed glass vials (100 seeds per vial) and immersed in polyethylene glycol-water (v/v) in controlled-temperature baths (Guy

Table 4. 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	Fig. 2
		b_0	b_1	b_2	b_3	b_4	b_5		
G	5	28.7	13.6	-0.2	-0.92			0.50	A
	15	24.4	11.1	1.0	-0.63	-0.04	-0.01	0.68	B
	25	24.5	9.7	1.3	-0.74	-0.06	-0.02	0.84	C
T_{50}	5	17.6	-2.9	0.03	0.26			0.69	D
	15	22.2	-3.9	-0.2	-0.31	0.02	0.002	0.90	E
	25	9.4	0.1	-0.05	0.40	0.06	0.01	0.90	F
$T_{90} - T_{10}$	5	9.8	0.24	-0.02			0.001	0.66	G
	15	10.0	0.19	-0.01			0.001	0.81	H
	25	16.1	-1.32	-0.01	-0.12	0.01		0.85	I

and Carter, 1984) for 7 days at 10, 5, 0, -5, -10, -15, or -20°C. Bath temperatures were lowered 3C/h to final temperatures, kept for 7 days, then increased 4C/h to 10°C. Following low- or subzero temperature treatment, seeds were germinated at constant 15C as described. Daily germination counts were made of seeds with radicle protrusion through the testa, and total germination data were analyzed using the least significant difference at $P = 0.05$.

Temperature and relative humidity interactions during seed storage. Immediately after harvest, seeds were cleaned and dusted with captan before storage at 11%, 33%, 52%, or 75% RH and 5, 15 or 25C for 3, 6, or 9 months. Humidity treatments were achieved as described above, except saturated lithium chloride, magnesium chloride, magnesium nitrate, and sodium chloride solution were used to achieve 11%, 33%, 52%, or 75% RH levels, respectively. Incubators with refrigeration and heating capacities maintained constant 5, 15, or 25C during seed storage. Seeds were germinated in petri dishes at constant 15C in darkness. Analysis of variance and response surface regression procedures were used to analyze the data for G, T_{50} , and $T_{90} - T_{10}$. Within each storage temperature, a randomized complete-block design was used with a factorial treatment structure (4×3) between RH and storage period.

Temperature during germination of tickseed influenced G, T_{50} , and $T_{90} - T_{10}$. At constant 15C, G was higher, while days to T_{50} and between $T_{90} - T_{10}$ were lower than at other constant temperatures (Table 1). The total germination percentage trend line for temperature was similar to the linear, quadratic, and cubic, while trend lines for days to T_{50} and days between $T_{90} - T_{10}$ were similar to the quadratic (Table 1).

Seeds germinated at constant 15C generally had higher G, fewer days to T_{50} , and a shorter span of days than at alternating 12-h cycles (Table 2). However, alternating 15/25C and constant 15C had similar numbers of days to T_{50} and $T_{90} - T_{10}$. In both temperature studies, 48% to 51% germination was achieved, indicating that temperature may not cause low total germination of coreopsis as previously reported (Samfield et al., 1990).

In a preliminary study, seeds were scarified in concentrated sulfuric acid (17 N) for 30 sec washed for 10 min, and vacuum-dried for 1 h. Scarified and nonscarified seed

were germinated at constant 15C as previously described. Similar G and days to T_{50} were found for scarified and nonscarified seed (data not presented). Soaking nonscarified seed for 6 h in 1000 ppm GA₃ or 1000 ppm ethephon, alone or in combination, resulted in higher G and fewer days to T_{50} and between $T_{90} - T_{10}$ (Table 3). Gibberellins and ethylene are phytohormones that have significant activity in seed physiology (Bewley and Black, 1985). Exogenous applications of GA₃ and ethephon have been reported to overcome the physiological dormancy of various seeds and to stimulate the germination of seeds with dormant embryos (Hartmann et al., 1990). Soaking seeds in 25 ppm kinetin had no significant effect on total germination and no effect on the rate of germination, nor did kinetin enhance germination in combination with GA₃ or ethephon (Table 3).

Lowering the temperature lengthens the storage life of seeds, which generally offsets the adverse effect of high seed moisture content (Hartmann et al., 1990). Harrington (1972) reported that seed storage life doubles with each temperature decrease of 5C between 44C and 0C in storage. Our results revealed that seeds of tickseed were cold-tolerant and total germination percentages increased following storage at 10C to -5C and declined after -10 to -20C storage (Fig. 1).

Total germination percentages of seed increased as storage durations at 5C increased at all relative humidities, achieving 75% germination at 10% to 20% RH after 6.5 to 8 months (Fig. 2A). Highest total germination at 15C was after seed storage at 20% to 35% RH, with 80% germination after 7 months of storage. Total germination at 15C declined after storage at relative humidities >50% (Fig. 2A-C). The maximum germination was 60% for seed stored at 25C, and germination rapidly declined when seeds were stored at >50% RH (Fig. 2C).

The fewest days to 50% of final germination resulted after 5 to 6 months of storage at 5 or 15C (Fig. 2D-F). Seeds stored at 5C and 10% to 20% RH or 10% to 40% RH at 15C had T_{50} of 10 days. Storing seeds at 5 or 15C for periods longer or shorter than 5 to 6 months increased days to T_{50} , as did >50% RH (Fig. 2 D and E). The largest delay in germination T_{50} was after seed storage at 25C and 10% RH. Increasing the 25C

storage at 10% RH from 3 to 9 months lengthened the T_{50} from 15 to 35 days (Fig. 2F).

The span in days between 10% and 90% germination lengthened as storage temperatures of seeds increased from 5 to 25C (Fig. 2 G-I). The trends were similar at all temperatures, with more days required for the $T_{90} - T_{10}$ span as RH and seed storage durations increased.

These studies provide information for increasing the total germination and promoting earlier and more-uniform germination of *C. lanceola* seed. Several prior studies were conducted to better understand the factors contributing to the low total germination of this species. The seed was very tolerant of desiccation, with no reduction in total germination when seed with 13% moisture content at harvest was dried to 5.2% (data not presented). Khan (1971) reported that cytokinins can offset the effect of chemical inhibitors in seeds. Soaking seeds for 6 h in 25 ppm kinetin caused no significant enhancement of total germination, indicating fresh seed had only low levels of chemical inhibitors. Seed recently harvested had much higher total germination after soaking for 6 h in 1000 ppm GA₃ or 1000 ppm ethephon. Ethylene is a natural germination-promoting substance for seeds of many plant species (Hartmann et al., 1990). Gibberellins comprise the class of hormones directly implicated in the control and promotion of seed germination (Khan and Samimy, 1982). Our results indicate that, at harvest, tickseed possibly has a deficiency in gibberellin, since additions of GA₃ or enhancement by ethephon treatment increased germination. Zeng and Khan (1984) report that gibberellin and ethephon application responses for improving the germination and seedling emergence of lettuce (*Lactuca sativa* L.) are remarkably similar.

Our results indicate that tickseed total germination percentages can be increased from 44% to ≈80% by a presowing treatment of seed in 1000 ppm GA₃ + 1000 ppm ethephon for 6 h or storage of seed at 5 or 15C and 20% to 30% RH for 6.5 to 9 months. However, growth regulator-treated seed had shorter T_{50} and $T_{90} - T_{10}$ than at the best storage treatment. Bewley and Black (1982) reported that increased total germination after storing the seeds of many genera at ≤ 15C resulted from the increased production of endogenous gibberellin and cytokinin, either during chilling or after transferring chilled seeds to recommended temperatures. Seeds stored at 25C achieved highest total germination of 60% at 10% to 40% RH, indicating that seeds benefited very little from storage at this temperature.

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