Evaluation of Temperature and Moisture Content during Storage on the Germination of Flowering Annual Seed

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Additional index words. relative humidity, germination

Abstract. Various combinations of temperature and moisture contents were used in evaluating the seed storage of nine genera of annual flowers. Relative humidity (RH) levels of 11%, 32%, 52%, and 75% provided wide ranges in seed moisture during storage at 5, 15, and 25C. At each temperature, total germination percentages (G) generally declined as seed moisture content increased during storage. The seed moisture range giving the highest G after 12 months of storage was determined for each temperature and plant genus. For all genera, seed moisture contents during storage increased as storage temperatures increased at constant RH levels. Moisture contents at 25C storage were 37%, 34%, 29%, and 20% higher than at 5C when RH levels were at 11%, 32%, 52%, and 75%, respectively.

The effects of temperature and relative humidity (RH) during storage on the aging of seed have been the subject of several reviews (Barton, 1961; Justice and Bass, 1978; Tilden and West, 1985). One general tenet is that the drier and cooler the storage environment, the longer orthodox seeds retain the ability to germinate. Stanwood and Bass (1981) found subzero storage temperatures, even at low seed moisture, cause structural deformations of cells that can cause reduced seed viability or performance. Also, seeds stored excessively dry may have accelerated deterioration rates. Decreased germinability for seeds of celery (*Apium graveolens* L. 'Dulce'), pepper (*Cap-sicum annuum* L. 'Jupiter'), and eggplant (*Solanum melongena* L. 'Esculentum') occurred as the moisture content during storage declined from 4% to 1% (Nutile, 1964). Touzard (1975) reported that, during storage, aster [*Callistephus chinensis* (L.) Nees] seed could tolerate 1% moisture, but total germination of *Primula obconica* Hance. and *Salvia splendens* seed rapidly declined when moisture content was <4%. Harrington (1973) stated

that moisture stresses occurring during excessive seed drying cause accelerated aging by compounding the degeneration of cellular membranes and the formation of microscopic cracks in seedcoats.

High levels of moisture in seeds during storage are the primary cause of accelerated aging of seeds. The hygroscopic nature of seeds allows the moisture content to be in equilibrium with the ambient RH in the storage container. Bewley and Black (1982) reported that seeds stored at high moisture levels had increased enzymatic activity and respiration, which hastened physiological deterioration. Storage temperature is known to modify the rate of seed physiological deterioration at unfavorable seed moisture contents (Hartmann et al., 1990), but to our knowledge, the extent of the effect of temperature has not been clearly documented. Our research objectives were to compare germination percentages for nine genera of flowering annuals after 12 months of storage over ranges of seed moisture content and temperature.

Seed handling and germination procedures. Seeds of nine genera of annual flowering plants were used in this study (Table 1). All seeds were received between 6 and 11 weeks from harvest and placed under the storage treatments of the study within 3 weeks of receipt. Seeds were dusted with 3 α ,4,7,7 α -tetrahydro-2-[(trichloromethyl)thiol]-1H-isoin-dole-1,3 (2H)-dione (captan) when received. Each treatment consisted of four 100-seed replications. After storage, the seeds were germinated in individual 9-cm petri dishes on Anchor blue blotter paper 100 (Anchor Paper Co., Charlotte, N.C.) moistened with 6 ml of distilled water when not otherwise designated. Seeds were germinated in incubators (Stults Scientific Engineering Corp., Springfield, Ill.), with each species receiving the recommended constant temperature and light or darkness. Germination counts were made daily. Seeds with radicles protruding through the testa were considered to have germinated in calculation of total percent germination (G).

Temperature $\times$ RH interactions during seed storage. Seeds were stored at 11%, 32%, 52%, or 75% RH and 5, 15, or 25C for 12 months. Specific humidities were achieved by placing 50 ml of saturated lithium chloride, magnesium chloride, magnesium nitrate, or sodium chloride solutions in the bottom of 15 $\times$ 2.5-cm petri dishes, with seeds on wire screens supported by segments of tubing 1 cm above the chemical (Copeland, 1976). The petri dishes were sealed with parafilm and placed in incubators with refrigeration and heating capacities maintaining constant temperatures during storage. After storage, seeds were germinated at the recommended conditions for each genus. Analysis of variance and response surface regression procedures were used to analyze the data for total germination. The design was a split-plot with storage temperature as the main-plot factor and RH as the subplot factor.

Measurement of seed moisture content during storage. When seed of each species was received, the moisture content of seed from the packet was determined by weighing four 100-seed lots, which were dehydrated in forced-draft ovens at 100C for 48 h before being reweighed after cooling. The weights of an additional eight 100-seed replications were measured before and after storage for 12 months at each temperature–RH treatment for all plant

Table 1. Plant species and cultivars of seeds stored 12 months at various temperatures and relative humidities and the moisture content for seeds in the packet when received.

Common	Flowering annuals in study		Moisture (%)
	Scientific	Cultivar	
Ageratum	<i>A. houstonianum</i> Mill.	Blue Blazer	6.2
Delphinium	<i>D. xcultorum</i> Voss	Magic Fountain Lavendar	7.1
Geranium	<i>Pelargonium xhortorum</i> L.H. Bailey	Scarlet Eye Orbit	7.0
Impatiens	<i>I. wallerana</i> Hook. f.	Accent Orange	3.4
Marigold	<i>Tagetes erecta</i> L.	Aurora Light Yellow	4.8
Pansy	<i>Viola xwittrockiana</i> Gams.	Majestic Giant Yellow	5.1
Phlox	<i>P. drummondii</i> Hook.	Light Salmon	7.4
Salvia	<i>S. splendens</i> , F. Sellow ex Roem & Schult.	Red Hot Sally	6.9
Vinca	<i>Catharanthus roseus</i> (L.) G. Don	Tropical Rose	7.4

Received for publication 5 Dec. 1994. Accepted for publication 24 Mar. 1995. Accepted for publication 24 Mar. 1995. Florida Agricultural Expt. Station Journal series no. R-04267. This research was sponsored partially by a grant from the Bedding Plants Foundation, Lansing, Mich. Seeds were donated by The Ball Seed Co., West Chicago, Ill.; Goldsmith Seeds, Gilroy, Calif.; Sakata Seed America, Salinas, Calif.; Sluis and Groot, Enkhuizen, Holland; and Waller Flowerseed Co., Guadalupe, Calif. 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.

genera. Then, four 100-seed replications from each treatment were dehydrated to determine seed moisture content; the remaining four replications were germinated as previously described.

Analyses of germination data. The effects of storage temperature and seed moisture content on the G of each of the nine species of seed were modeled by fitting the following polynomial regression equation to the germination percentages (Table 2): Average $G = b_0 + b_1T + b_2M + b_3T \times M + b_4T^2 + b_5M^2 + b_6T \times M^2$. T represents storage temperature; M, seed moisture; b_0 , b_1 , and b_2 , the intercept or average percent germination when $T = M = 0$ and the linear effects of temperature and seed moisture; b_4 and b_5 , the quadratic effects of T and M, respectively; and b_3 and b_6 , the linear $\times$ linear and linear $\times$ quadratic interaction effects of T and M, respectively.

Results

No seeds germinated at the high temperature (25C)–high moisture (75% RH) combination for any of the nine genera (Table 2). At 15C and 75% RH, G was <25% for all genera, except ageratum, geranium, and phlox. To model these large declines in G values across all levels of seed moisture and temperature would require including higher-order terms of the form $b_7T^2 \times M^2$, b_8M^3 , and $b_9T^2 \times M^3$ in the polynomial regression equation or using more complicated nonlinear regression equations to model the extremely complicated-shaped germination surfaces. Opting to use terms of degree three or lower in the polynomial regression equation to model the germination surfaces prompted us to consider only those temperature–seed moisture combinations during storage that permitted >25% germination. Thus, average G values <25% were not included in the data analyses that produced the regression equations (Table 3).

Among the higher-order coefficient estimates (b_3 , b_4 , b_5 , and b_6) in the fitted polynomial regression equations, only those having values significantly ($P < 0.05$) different from zero are listed (Table 3). Associated with each fitted regression equation is an approximate coefficient of determination value (R^2_{Ave}). The value of R^2_{Ave} is only approximate because average G values across four replications were fitted rather than individual G values. We recognize that the value of the coefficient of determination (R^2) is inflated when averages rather than individual observations are fitted (Cornell and Berger, 1987). The approximate R^2_{Ave} values in Table 3, ranging from 0.913 to 0.977, were used to compare the fits of the regression equations across the nine genera of seed, where the higher the R^2_{Ave} value, the better the fit.

The contour plots (Fig. 1) of the estimated average G surfaces were generated by the equations listed in Table 3; the regions enclosed by the solid lines for each genus are the data regions. These data regions are defined by the values of seed moisture content and storage temperature (Table 2). The extra region, outside the solid boundary but contained within

Table 2. Average moisture content and percent total germination (G) of bedding plant seeds stored at various temperature and relative humidities for 12 months. Averages of four 100-seed replications.

Genus	Storage temp (°C)	Seed moisture content (%)				Total G (%)			
		Storage relative humidity (%)				Storage relative humidity (%)			
		11	32	52	75	11	32	52	75
Ageratum	5	2.0	3.3	4.7	7.3	96	96	95	93
	15	2.3	4.1	5.2	8.1	94	86	96	52
	25	2.7	5.0	5.9	9.2	93	95	81	0
Delphinium	5	4.9	6.8	8.3	11.1	44	71	63	40
	15	6.3	7.4	9.1	12.7	61	60	57	0
	25	6.9	7.9	9.5	13.7	51	43	31	0
Geranium	5	5.1	6.0	8.3	13.2	88	86	89	85
	15	6.4	7.0	9.0	14.2	87	90	91	73
	25	6.8	7.3	10.5	15.8	91	91	84	0
Impatiens	5	2.2	2.5	3.2	5.6	87	96	92	82
	15	3.2	4.1	5.0	7.5	92	96	90	3
	25	3.4	4.9	6.0	7.8	92	96	50	0
Marigold	5	3.5	5.2	5.8	9.4	92	96	95	87
	15	3.9	6.0	6.3	9.8	92	91	94	14
	25	4.9	6.9	7.4	10.2	95	83	82	0
Pansy	5	2.7	4.0	4.5	8.2	90	87	84	75
	15	3.0	5.9	6.1	10.0	75	84	88	20
	25	4.2	5.9	6.8	10.7	77	83	79	0
Phlox	5	6.5	7.7	9.7	11.2	97	95	96	81
	15	6.7	8.0	10.0	11.6	91	89	83	36
	25	7.0	8.2	10.4	12.0	85	88	75	0
Salvia	5	3.4	5.0	7.2	10.6	69	73	71	26
	15	4.1	5.8	7.4	11.5	69	77	63	0
	25	4.3	6.0	7.5	12.3	69	70	17	0
Vinca	5	3.0	5.1	6.7	9.2	90	93	91	33
	15	3.6	5.8	7.1	9.5	89	93	91	24
	25	3.8	5.7	7.5	9.7	89	89	89	0

Table 3. Average total germination (ATG) after 12 months of storage modeled as a function of seed storage temperature (T) and seed moisture content (M) with the following regression equation: $ATG = b_0 + b_1T + b_2M + b_3T \times M + b_4T^2 + b_5M^2 + b_6T \times M^2$.

Annual	Coefficient estimates							R^2_{Ave}
	b_0	b_1	b_2	b_3	b_4	b_5	b_6	
Ageratum	115.14	-3.45	-10.47	1.87	---	1.22	-0.24	0.920
Delphinium	-100.24	1.61	43.11	-0.34	---	-2.66	---	0.937
Geranium	81.23	-1.13	1.19	0.35	---	-0.04	-0.02	0.952
Impatiens	123.02	-12.20	-10.76	5.86	---	0.69	-0.67	0.977
Marigold	64.18	0.97	10.06	-0.22	---	-0.75	---	0.932
Pansy	128.20	-6.03	-14.95	2.42	-0.03	1.03	-0.20	0.947
Phlox	58.89	0.84	6.59	-0.11	---	-0.34	---	0.913
Salvia	60.00	-9.24	0.68	4.38	-0.07	0.14	-0.43	0.960
Vinca	21.08	-1.37	34.06	0.23	---	-3.63	---	0.960

the dashed line, is the seed moisture region where G is <25% after storage. For example, ageratum seed stored 12 months at 5C with moisture contents ranging from 2.0% to 7.3% had G >25%, as did seed stored at 15C at moisture contents ranging from 2.3% to 8.1% (Table 2). Ageratum seeds stored at 25C with moisture contents ranging from 2.7% to 5.9% had G >25%, but seeds with a moisture content of 9.2% during storage did not germinate (G = 0). Thus, the value of G = 0 at the temperature–seed moisture content combination of 25C and 9.2% moisture content during storage was not included in the data set used to obtain the fitted regression equation for ageratum (Table 3).

G values varied greatly with each genus when compared across the various storage temperature–seed moisture combinations and across the nine genera. Although the maximum G for most of the genera followed stor-

age at temperatures of 5 and 15C with seed moisture contents <10%, storage temperature had little or no effect with other genera. Because the optimum seed moisture range for each genus varied with storage temperature and the optimum temperature and was not the same for all genera, recommended storage conditions for the nine genera can only be made by focusing on the germination results of each genus separately.

Ageratum. Seed stored 12 months at 5C and 11% to 75% RH had moisture contents ranging from 2.0% to 7.3% (Table 2) and G ranging from 93% to 95% (Table 2 and Fig. 1). Seed with 2.3% to 5.2% moisture content during storage at 5C and 15C had similar G. In fact, storage temperature had little or no effect on G for seeds with moisture contents ranging from 2.0% to 5.2% (Fig. 1). Seed with high moisture content (>8%) stored at 15 or 25C

had much lower G (52% and 0%, respectively) than seed stored at 5C with 7.3% moisture content (G = 93%). The effect of increasing seed moisture content on G differed at the three storage temperatures, where values of G behaved curvilinearly at 15 and 25C. At 5C, G remained constant (Tables 2 and 3; Fig. 1).

Delphinium. During 12 months of storage at 5C and 11% to 75% RH, seed moisture contents ranged from 4.9% to 11.1% (Table 2). Highest G (55% to 65%) coincided with seed having 6.8% to 8.3% moisture content after 5C storage, with G declining as seed moisture levels deviated from this range or as storage temperature increased (Fig. 1). At 11% RH, seed at 15C with 6.3% moisture content had higher G than seed at 5C with 4.9% moisture (61% compared to 44%) (Table 2), but in general, G was lower at 15 and 25C than at 5C for seed having high (>10%) moisture contents. At 25C, highest G (51%) occurred with seed having a moisture content of 6.9%, with germination declining from 43% to 0% as seed moisture levels during storage increased from 7.9% to 13.7% (Table 2). G values behaved curvilinearly with varying seed moisture contents, where the greatest effect of seed moisture on G occurred at 5C (Fig. 1 and Table 3).

Geranium. G values varied little (85% to 89%) after storage for 12 months at 5C for seeds having moisture contents ranging from 5.1% to 13.2% (Table 2). At 15 and 25C, G values were high (87% to 91%), with seeds having moisture contents ranging from 6.4% to 9.0% and from 6.8% to 7.3%, respectively. At 15C, G declined from 91% to 73% for seeds with moisture contents ranging from 9.0% to 14.2%, respectively; at 25C, G declined from 91% to 84% for seeds with an increase in moisture content from 7.3% to 10.5%. A further increase in seed moisture content to 15.8% at 25C resulted in no germination.

Impatiens. Highest G (92% to 96%) occurred after storage at 5C for seeds having 2.5% to 3.2% moisture content, at 15C for seeds having 3.2% to 4.1% moisture content, and at 25C for seeds having 3.4% to 4.9% moisture content (Table 2). G declined for seeds having moisture contents of 5.6%, 5.0%, and 6.0% when stored at 5, 15, or 25C, respectively, especially at 15 and 25C. Very few seeds with moisture contents >7.5% germinated after 12 months of storage at 15 or 25C (Table 2).

Marigold. Total germination was affected more by extreme changes in seed moisture content than by storage temperature. Similar G (91% to 96%) resulted from seeds having moisture contents ranging from 3.5% to 6.3% at the three storage temperatures (Table 2). Seeds with 9.4% moisture content stored at 5C had G reduced to 87%, and seeds with 9.8% moisture content stored at 15C had only 14% G. G was 83% and 82% at 25C with seeds having 6.9% and 7.4% moisture contents, respectively. Seeds with a 10.2% moisture content failed to germinate.

Pansy. Seed stored at 11% RH and 5C had a lower seed moisture content (2.7%) and higher G (90%) than seeds stored at 15 or 25C with 11% RH. G values were similar (77% to

88%) among the three storage temperatures when seed moisture ranged from 4.0% to 6.8%. Seed with 8.2% moisture content stored at 5C had lower G (75%) than seed with lower moisture contents stored at 5C. Only 20% germination was achieved from seed after storage at 15C and with 10% moisture content. Seed stored at 25C and 10.7% moisture content failed to germinate after storage.

Phlox. After 12 months of storage at 5C, seed with 6.5% to 9.7% moisture contents had similar G (95%), but G declined to 81% for seed having 11.2% moisture content (Table 2). G from seeds having 6.7% to 8.0% moisture contents during storage at 15C were lower (91% to 89%) than for seed with similar moisture contents stored at 5C. Only 36% of the seeds having a moisture content of 11.6% and stored at 15C germinated (Table 2). At 25C, seed moisture contents during storage were similar to those at 15C at the four RH levels, but G generally was lower at 25C for seed having moisture contents >10%. Seeds with a moisture content of 12% and stored at 25C failed to germinate.

Salvia. Total germination was similar (70%) for seeds stored 12 months at 5C and moisture contents ranging from 3.4% to 7.2%,

for seeds having moisture contents from 4.1% to 5.8% and stored at 15C, and for seeds with moisture contents from 4.3% to 6.0% stored at 25C. Seed moisture contents >7.2% at 5C, 5.8% at 15C, or 6.0% at 25C caused reduced germination. Seeds having 11.5% and 12.3% moisture contents during storage at 15 and 25C, respectively, failed to germinate.

Vinca. Storage temperature had minimal effect on G (90%) for seeds with moisture contents ranging from 3.0% to 7.5%. Seed with moisture contents >9% had lower G (33% and 24%) after storage at 5 and 15C, respectively, and none germinated after storage at 25C.

Discussion

Generally, at 5, 15, or 25C, G declined as the seed moisture content increased during storage (Table 2). Two exceptions were ageratum seed stored at 5C (2.0% to 7.3% seed moisture contents) and geranium seed stored at 5C and 5.1% to 13.2% moisture. Highest G (71%) for delphinium seed occurred after 5C storage at 6.8% moisture content, with reduced G after storage at 4.9% or >8.3% moisture contents. Impatiens seed stored at 5C with

Fig. 1. Estimated total germination percentages (G) for nine species of annual flowering plants after 12 months of storage at 5, 15, or 25C and 2% to 14% seed moisture. Within the areas bounded by solid lines, G is >25%; in areas outside the solid lines, but within dashed lines, G is <25%.

2.5% to 3.2% moisture contents had 96% and 92% G, respectively (Table 2). Seed moisture content probably determined the rate of food metabolism during storage, with severe desiccation preventing metabolism. Petruzzelli and Taranto (1984) reported that decarboxylation and transamination reactions begin in wheat (*Triticum aestivum* L.) at 6% moisture content, and at >15% moisture content, metabolism is substantial. Cellular mitochondria become better organized at seed moisture contents >8%; <3% moisture mitochondria appear swollen (Barton, 1953). Harrington (1973) reported that seeds stored excessively dry frequently have accelerated deterioration rates. Hallam et al. (1973) found that seeds of grain crops had increasing rates of gaseous exchange and greater activity of respiratory enzymes during storage at >8% moisture content.

The seed moisture range giving the highest G after 12 months of storage was determined for each temperature and plant genera (Table 2). The extent of the optimum seed moisture range varied with the storage temperature for each genera. For example, *ageratum*'s highest germination (93% to 96%) after storage at 5C was with seeds having 2.0% to 7.3% moisture contents, at 15C with seeds having 2.3% to 5.2% moisture contents, and at 25C with seeds having 2.7% to 5.0% moisture contents. Therefore, seeds of this genera store equally well for 12 months at 5 to 25C if provided the proper seed moisture range. Seeds stored 12 months at 75% RH had higher G at 5C than at 15 or 25C. The large differences in seed moisture contents among genera (Table 2) stored at the same temperature and RH levels probably

were due to different kinds of food storage reserves within the seeds (Barton, 1941).

For seeds of the genera on trial, the percent moisture content during storage increased as storage temperatures increased at each constant RH level. Across the nine genera, seed moisture levels at 25C storage were 37%, 34%, 29%, and 20% higher on average at 11%, 32%, 52%, or 75% RH levels, respectively, than the average moisture contents at the same RH levels at 5C storage temperature. Hartmann et al. (1990) stated that the control of seed moisture during storage is probably the most important factor in seed longevity. The cause of lower G after 25C seed storage could be from accelerated rates of metabolism and seed aging at higher moisture contents.

The results of this study show that high G for each species can be achieved after 12 months of storage at 5, 15, or 25C, providing that seed moisture content is in the optimum range during storage. Storage at 5C may reduce the utilization rate of essential substances, including plant growth regulators, that are needed to promote germination. For seeds of annual flowering plants that are stored commercially for <1 year, 15 or 25C storage may be adequate, but for longer storage, we recommend temperatures <15C.

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