Germination and Storage of Vinca Seed Is Influenced by Light, Temperature, and Relative Humidity

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Abstract. Light, temperature, relative humidity (RH), and GA₃ affect vinca [*Catharanthus roseus* (L.) G. Don] seed storage and/or germination. GA₃ failed to increase the germination percentage in darkness but significantly increased the percentage in continuous light. Similarly, GA₃ treatment reduced both the number of days required to achieve 50% of the final germination percentage (T₅₀) and the span between 10% and 90% germination (T₉₀ - T₁₀) for seeds in light, but not in darkness. Germination percentages were maximal and about equal at 25, 30, or 35°C in darkness; germination was lowest below 25°C. Germination T₅₀ and T₉₀ - T₁₀ required the fewest days between 25 and 35°C. Reducing seed moisture from 9.9% to 3.9% increased the T₅₀ from 2.4 to 3.0 days but failed to change germination percentages. Germination percentage declined linearly as seed storage temperatures were reduced from 5 to -20°C, whereas days to T₅₀ increased. Seed storage for 12 months without reduction in germination percentage was possible at 5°C and 11%, 33%, or 52% RH, but storage at 75% or 95% RH for periods exceeding 1 month reduced germination. Seeds stored at 33% or 52% RH required fewer days to T₅₀ than did seeds stored at 11%, 75%, or 95% RH. Chemical name used: gibberellic acid (GA₃).

Vinca, a perennial, is generally grown as an annual bedding plant. Propagation is by seed, with optimal germination temperature ranging from 20 to 27°C (Mastalerz, 1976; Styer and Laffe, 1990). Carlson (1986) reported that germination ceases when the temperatures fall below 15°C or the medium becomes slightly dry. Vinca seed is sensitive to light during germination. Mastalerz (1976) and Ball (1991) recommended that the seed be covered lightly with the sowing medium and kept in darkness during germination. Bewley and Black (1982) classified plant species in terms of light sensitivity during germination, according to whether dormancy was terminated or germination was inhibited by light. Mayer and Poljakoff-Mayber (1979) reported that the light response of seeds from sensitive plant species increased with time during imbibition, with lettuce (*Lactuca sativa* L.) reaching maximum sensitivity about 1 h after completing imbibition. Bewley and Black (1982) found that periods of light sensitivity during germination were similar for seeds of species requiring light or darkness.

Our research objectives were to determine the effects of relative humidity (RH) during seed storage and of temperature and light on germination.

Temperature for germination. Vinca seeds of 'Grape Cooler', 'Peppermint Cooler', and 'Pretty in Rose', harvested 17 Dec. 1989 (Denholm Seeds, Lompoc, Calif.), were received 26 Dec. 1989 and dusted with 3 α ,4,7,7 α -tetrahydro-2-[(trichloromethyl)thio]-1H-isoindole-1,3(2H)-dione (captan). The treatments contained four 100-seed replicates, with seeds of each germinated in in-

dividual 9-cm petri dishes on a double layer of Whatman No. 1 filter paper moistened with 5 ml distilled water (DW). Seeds were germinated in dark incubators at constant 15, 20, 25, 30, or 35°C, and germination counts were made in darkness with 0.21 mol·m⁻²·s⁻¹ of photosynthetically active radiation from a green lamp. Temperature-controlled incubators (model CB-1; Stults Scientific Engineering Corp., Springfield, Ill.) were used in the study. Daily germination counts were made of seeds with visible radicle protrusion through the testa. Total germination percentage (G), days to 50% of the final germination percentage (T₅₀), and germination span, in number of days elapsed between 10% and 90% germination (T₉₀ - T₁₀), were calculated in all studies, as recommended by Furutani et al. (1985). The design was a split plot; data were tested by analyses of variance (ANOVA), and trends in temperature were determined through orthogonal contrasts (Steel and Torrie, 1960).

Gibberellin and germination in light or in darkness. Seeds of 'Grape Cooler', 'Peppermint Cooler', and 'Pretty in Rose' were placed for 6 h in 9-cm petri dishes on two Whatman No. 1 filter papers wetted with 10 ml of DW or 10 ml of 1000 ppm GA₃. After imbibition, all seeds were removed, blotted dry, and transferred to petri dishes with filter papers moistened by 5 ml DW. Treatments (four replications of 100 seeds) imbibed in DW or in GA₃ were germinated with 24 h of irradiation (60-W incandescent lamps at 75 μ mol·m⁻²·s⁻¹) or in darkness at 25°C. Seeds with emerged radicles were counted daily for 21 days; G, T₅₀, and T₉₀ - T₁₀ were calculated. The design was a split plot, and ANOVA were conducted for light and dark as the main plots and GA₃ treatments as the subplots. Separate analyses were made for each cultivar. Differences among treatments were determined using Fisher's LSD procedure (Milliken and Johnson, 1984) at P = 0.05.

Seed moisture content. Four 100-seed lots of the freshly harvested 'Grape Cooler' vinca were weighed, dehydrated at 105°C for 48 h, and reweighed after cooling to determine the

Table 1. The effect of gibberellic acid (GA) on vinca seed germination in light or in darkness at 25°C. A separate analysis was conducted with the data from each cultivar. Data are the means of 400 seeds.

Cultivar	Treatment	Germination ^z		
		Total (%)	T ₅₀ ^y (days)	Span ^x (days)
Grape Cooler	Dark	100 a	1.9 b	1.8 c
	Dark + GA	97 a	2.0 b	2.0 c
	Light	71 b	4.1 a	5.2 a
	Light + GA	87 a	3.8 a	4.6 b
Peppermint Cooler	Dark	96 a	1.9 c	2.0 bc
	Dark + GA	98 a	1.7 c	1.6 c
	Light	75 b	3.4 a	3.3 a
	Light + GA	96 a	2.2 b	2.4 b
Pretty in Rose	Dark	81 a	2.4 b	2.8 b
	Dark + GA	82 a	2.7 b	2.8 b
	Light	65 b	3.3 a	3.9 a
	Light + GA	83 a	2.7 b	2.8 b

^zMean separation for each cultivar within columns by Fisher's LSD procedure, P = 0.05.

^yDays to 50% of final germination.

^xDays from 10% to 90% of final germination.

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Fig. 1. Temperature influence on germination percentage (G), days to 50% of final germination (T_{50}), and span in days between 10% and 90% germination ($T_{90} - T_{10}$) for three cultivars of vinca. The orthogonal polynomial contrast used to determine the trends in temperature were:

(A) Grape Cooler:

$$\hat{G} = -56.3 + 10.5 \times \text{Temp} - 0.18 \times \text{Temp}^2; R^2 = 0.69$$

$$\hat{T}_{50} = 30.3 + 2.0 \times \text{Temp} + 0.04 \times \text{Temp}^2; R^2 = 0.98$$

$$\hat{\text{Span}} = 18.6 - 1.2 \times \text{Temp} + 0.02 \times \text{Temp}^2; R^2 = 0.83$$

(B) Peppermint Cooler:

$$\hat{G} = -74.9 + 11.0 \times \text{Temp} - 0.17 \times \text{Temp}^2; R^2 = 0.87$$

$$\hat{T}_{50} = 22.8 + 1.4 \times \text{Temp} + 0.02 \times \text{Temp}^2; R^2 = 0.89$$

$$\hat{\text{Span}} = -1.2 + 1.0 \times \text{Temp} - 0.06 \times \text{Temp}^2; R^2 = 0.89$$

(C) Pretty in Rose:

$$\hat{G} = -147.5 + 16.6 \times \text{Temp} - 0.30 \times \text{Temp}^2; R^2 = 0.72$$

$$\hat{T}_{50} = 74.3 - 7.0 \times \text{Temp} + 0.23 \times \text{Temp}^2; R^2 = 0.98$$

$$\hat{\text{Span}} = -2.3 + 1.86 \times \text{Temp} - 0.11 \times \text{Temp}^2; R^2 = 0.89$$

Table 2. Influence of seed storage relative humidity and duration on the germination percentage and days to 50% of final germination of 'Grape Cooler' vinca.

Seed storage at 5C					
RH (%)	Seed moisture (%)	Months			
		1	4	8	12
<i>Germination (%)</i>					
11	4	88	90	91	93
33	6	92	93	89	94
52	8	94	91	92	88
75	11	90	84	68	13
95	15	73	28	11	0
Significance					
Moisture (M)	**				
Storage period (SP)	*				
M × SP	*				
<i>Days to 50% of germination</i>					
11	4	3.5	3.8	4.0	4.3
33	6	3.4	3.1	3.2	3.3
52	8	3.2	3.3	3.0	2.9
75	11	3.7	3.9	5.2	5.5
95	15	3.9	4.5	7.1	---
Significance					
Moisture (M)	**				
Storage period (SP)	**				
M × SP	*				

***Significant at $P = 0.05$ or 0.01 , respectively; data are the means of 400 seeds.

initial moisture content. Other treatments were weighed, placed in open 9-cm petri dishes, and dehydrated in forced-draft ovens at 40C for 1, 2, 4, 8, 12, 24, or 48 h. After dehydration, seeds were reweighed and immedi-

ately germinated, as previously described, at 25C in darkness. A split plot ANOVA was used, with duration of dehydration as the main plot and seed moisture as the subplot. Orthogonal polynomial contrasts were used to

determine seed moisture trends.

Storage temperature. Seeds were stored for 1 week in 15×2.5 cm petri dishes on wire screens supported by segments of tubing 1 cm above a chemical desiccant that reduced the 9.9% moisture content of freshly harvested 'Grape Cooler' seed to 7.2%. Constant 22% RH was maintained in sealed petri dishes by addition of 50 ml of saturated potassium acetate to the bottom of each dish (Copeland, 1976). The incubator was maintained at 15C during the week of reduced seed hydration. After dehydration, seeds were weighed, placed immediately into 10-ml glass vials, which were then sealed, and immersed in a controlled-temperature polyethylene glycol-water (v/v) bath (Guy and Carter, 1984) held at 5, 0, -5, -10, -15, or -20C. Bath temperatures were lowered 3C/h to final temperatures, held there for 7 days, then increased 4C/h to 10C. Following low-temperature treatment, seeds were germinated in darkness at 25C. Germination counts were made daily of seeds with radicle protrusion through the testa. Data were analyzed by ANOVA, and orthogonal contrasts were used to determine temperature trends (Steel and Torrie, 1960).

Relative humidity during and duration of seed storage. Freshly harvested seed of 'Grape Cooler' was dusted with captan and stored at 5C and various relative humidities for 1, 4, 8, or 12 months. Humidity treatments were achieved using saturated lithium chloride, magnesium chloride, magnesium nitrate, sodium chloride, or potassium nitrate, as described previously, to achieve 11%, 33%, 52%, 75%, or 95% RH levels, respectively. Four 100-seed replications were germinated in darkness at 25C immediately after storage at each duration and at each RH level, whereas another four replications were desiccated for 48 h in forced-draft ovens at 105C to determine moisture contents of stored seed. The design was a 4×5 factorial arranged in a randomized complete block, and data were analyzed by ANOVA.

Vinca seeds required high temperature and darkness for best germination (Fig. 1 and Table 1, respectively). Total germination percentages for 'Grape Cooler' and 'Peppermint Cooler' were similar at 25, 30, and 35C and for 'Pretty in Rose' at 25 and 30C. Contrasts were performed by comparing the average response at the high temperatures (25, 30, and 35C) for 'Grape Cooler' and 'Peppermint Cooler' and at 25 and 30C for 'Pretty in Rose' against the average response at the two lower temperatures (15 and 20C). For all cultivars, G at temperatures below 25C was significantly ($P < 0.01$) lower than at higher temperatures (Fig. 1). The number of days to achieve T_{50} at 25, 30, and 35C was significantly ($P < 0.01$) lower than below 25C for all cultivars (Fig. 1). The $T_{90} - T_{10}$ were significantly ($P < 0.01$) shorter for 'Grape Cooler' and 'Peppermint Cooler' at 25, 30, and 35C and for 'Pretty in Rose' at 25 and 30C than at the other temperatures (Fig. 1). These results indicate that vinca seed needs higher germination temperatures than the 20 to 25C recommended by Mas-

Fig. 2. Seed moisture content of 'Grape Cooler' vinca in relation to days to 50% of final germination (T_{50}). The regression equation was: $T_{50} = 4.19 - 0.39 \times S M^2 + 0.218 \times S M$; $R^2 = 0.60$.

Fig. 3. Storage of 'Grape Cooler' vinca seed at freezing temperatures in relation to (A) total germination percentages (G) and (B) days to 50% of final germination (T_{50}). The linear regression equations were: $G = 94.6 + 0.24 \times \text{Temp}$, $R^2 = 0.42$; $T_{50} = 2.7 - 0.02 \times \text{Temp}$, $R^2 = 0.45$.

talerz (1976) and Carlson (1986), and the temperature range is broader for highest total germination than that reported by Styer and Laffe (1990) and Ball (1991).

Vinca seed germination percentage was higher in darkness than in light, as also reported by Ball (1991). Total germination in darkness was not increased by the addition

of GA₃ during seed imbibition (Table 1). GA₃ significantly increased total germination percentages of seeds in continuous light, with GA₃ eliminating the need for darkness. Application of GA₃ had no effect on the T_{50} or $T_{10} - T_{50}$ of seeds germinated in darkness, but it did in light (Table 1). Germination in light lengthened the periods to T_{50} and $T_{10} - T_{50}$ for seeds of each cultivar, but GA₃ treatment of seeds shortened both germination periods in light relative to those in darkness for 'Pretty in Rose'. GA₃ treatment generally reduced the T_{50} and $T_{10} - T_{50}$ for 'Grape Cooler' and 'Peppermint Cooler' seed germinated in light, but more days were required for germination in light than in darkness (Table 1). The capacity of GA₃ to allow the full germination of light- or dark-requiring seeds under unfavorable light conditions was reported by Chen and Thimann (1966). Co-cucci et al. (1981) found that light inhibited the germination in dark-requiring *Phacelia tanacetifolia* Benth. seeds, but germination in light was promoted after seed pretreatment with acetone containing GA₃.

Seeds of 'Grape Cooler' tolerated desiccation, and no trend or difference occurred in G (89% to 93%) for seed moisture contents from 9.9% to 3.9% (data not presented). A quadratic trend was established when T_{50} values increased from 2.4 to 3.0 days as seed moisture levels declined (Fig. 2). Evidently, the level of seed desiccation during storage influenced the period required for germination. Justice and Bass (1978) report that the first indication of seed injury from low moisture content is delayed germination.

'Grape Cooler' seeds at 7.2% moisture content were cold-tolerant, but a linear increase in G occurred as storage temperature increased from -20 to 5C (Fig. 3A). Storage of seeds for longer than 7 days at any of the temperatures tested might reduce G further. A linear decrease in the number of days to T_{50} was found as seed storage temperatures increased (Fig. 3B). These results indicated that germination was delayed as seed storage temperatures declined. Sub-freezing temperatures are superior to higher temperatures for the long-term storage of seeds because of lower seed respiration (Justice and Bass, 1978). Barton (1932) reported that *Delphinium × cultorum* Voss and *Delphinium elatum* L. seed had no significant loss in total germination or vigor after storage for 5 years at -15C.

Significant differences were found for G and T_{50} for seed moisture (M) content during storage, the duration of the storage period (SP), and the interaction $M \times SP$. Seed storage for 12 months without reduction in G was possible at 5C and 11%, 33%, or 52% RH, but storage at 75% or 95% RH for periods exceeding 1 month reduced G (Table 2). The seed moisture percentages shown (Table 2) are the means for seeds removed at each humidity level after storage for 1, 4, 8, or 12 months. Seeds stored at 33% or 52% RH required fewer days to achieve T_{50} than did seeds stored at 11%, 75%, or 95% RH (Table 2).

Moisture in seeds is in equilibrium with the ambient relative humidity of the storage container. Seed moisture percentage at a given RH depends on the species storage reserves (Atwater, 1980). Seeds of vinca and of many other species are desiccation-tolerant and require low moisture levels for long-term storage. A 4% to 6% seed moisture content is considered favorable for the prolonged storage of these species (Hartmann et al., 1990). Bewley and Black (1982) reported that many species lose viability at moisture levels below 4%, causing reduced germination rates or delayed germination. The significant delay in vinca germination after storage at 11% RH probably was due to excessive seed desiccation (4% moisture). Com (1980) found that the harmful effects of excessively low seed moisture level during storage could be mitigated by slow rehydration with saturated water vapor before sowing.

Bradbeer and Pinfield (1967) reported that the maximum seed viability of many species occurred when seeds were stored at 20% to 25% RH. Our results showed that vinca seed stored at 33% and 52% RH had 6% and 8% moisture contents, respectively, and germinated significantly faster than at other levels, without a reduction in G. Use of 5C made storage possible at these moisture levels. A rapid reduction in G occurred when vinca seed was stored at 75% or 95% RH. Hartmann et al. (1990) reported that seeds stored at high moisture levels had increased enzymatic activity and respiration, which hastened physiological deterioration.

This study presents new information for improving the environment during storage and germination of vinca seed. Germination percentages were highest at 25 to 35C in darkness, temperatures considerably warmer than those previously reported. The research also confirmed the need for darkness during germination, but seed treatment with gibberellins made germination percentages in light similar to those in darkness. Hartmann (1990) reported that gibberellins can relieve photodormancy in lettuce seed by promoting germination without light; our results showed that for vinca gibberellins can replace the requirement for darkness. Several contributing factors were identified that affect the germination of vinca seed. Storing seed at high or low relative humidity (above 52% or below 11%) or at subzero temperatures, or germinating seed at unfavorable temperatures contributed to irregular embryo emergence.

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