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Effect of Storage Temperature and Seed Moisture on Germination of Stored Flowering Dogwood Seed[1]

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Abstract

Dogwood producers occasionally face a shortage of flowering dogwood seed. Storing excess seed during years when seed are abundant would allow growers to stabilize their seed supply. This study was conducted to evaluate the effects of seed moisture and storage temperature on the viability of stored flowering dogwood seed. Seed were collected in Fall 1999 and 2000, dried to 6, 10 and 14% moisture content, and stored at 22, 5 and –20C (72, 41 and –4F) for 1, 2 and 3 years. Following storage, seed were cold stratified and sown in a greenhouse. Percent germination and seedling dry weight were recorded. Seed stored at 22C (72F) quickly lost viability. At 5C (41F), seed moisture content was critical, with seed dried to 14% moisture content germinating poorly after 2 years and failing to germinate after 3 years in storage. In general, storage at –20C (–4F) was superior to storage at 5C (41F). Seed moisture content was not as critical at –20C (–4F) as it was at 5C (41F), but may become more important if length of storage is extended past 3 years. Based on the results of this study, it is recommended that seed be dried to 6 to 10% moisture prior to storage, stored in air-tight containers, and stored in a –20C (–4F) freezer.

Index words: *Cornus florida*; seed storage; seed viability.

Significance to the Nursery Industry

An adequate supply of seed is a necessity for production of flowering dogwood; however, growers occasionally face a seed shortage. The objective of this study was to develop a reliable method of storing flowering dogwood seed that will allow producers to overcome year-to-year fluctuations in seed production. Because seed moisture level and storage temperature are known to greatly influence the viability of stored seed, these two factors were examined. Seed were dried to 6, 10 and 14% moisture content and stored at room temperature (22C/72F), in a refrigerator (5C/41F) and in a freezer (–20C/–4F) for 1, 2 and 3 years. Following storage, seed were stratified to break dormancy and sown in a greenhouse. Seed stored at room temperature quickly lost viability. Moisture content was critical for seed stored in a refrigerator, with seed dried to 14% moisture content germinating poorly after 2 years and failing to germinate after 3 years in storage. In general, seed stored in a freezer germinated much better than that stored in a refrigerator. Based on the results of this study, it is recommended that dogwood seed be dried to 6 to 10% moisture content prior to storage, packaged into air-tight containers, and stored in a –20C (–4F) freezer. Use of these storage conditions should allow flowering dogwood seed to be stored for at least 3 years without a significant loss in viability and will stabilize dogwood seed supply.

Introduction

Flowering dogwood (*Cornus florida* L.) is one of the most popular landscape trees in the United States. Annual sales of *C. florida* exceed $26 million, ranking it fourth among flowering deciduous trees for U.S. sales (11). Approximately 100 cultivars have been described, representing a range of bract colors, inflorescence forms, plant habits, foliage colors and pest resistance (4, 9, 13, 14). Cultivars are primarily propagated by budding onto seedling rootstock (5, 10). In addition to the named cultivars, a considerable number of seed-propagated plants, listed as 'white' or 'pink' dogwoods, are produced and marketed by some growers.

The flowering dogwood fruit is a globular or ovoid drupe which contains a 2-celled stone (16). Since the stone usually has only one fully-developed seed, the term seed will be used throughout this paper in place of stone. Dogwood fruit are collected in fall when the flesh of the berry begins to soften (1, 2). Depending on the grower and time of seeding, the seed may or may not be removed from the fruit before planting. Some growers sow dogwood seed in seedbeds in midfall, while others wait and plant in early spring. If seed are to be sown in spring, they are cleaned shortly after collection in the fall and then stored in a moist, cool environment until planting. Dogwood seeds exhibit a high degree of dormancy and require stratification at 5C (41F) for 90 to 120 days (4).

An adequate supply of dogwood seed is a necessity for production of this economically important crop; however, growers have occasionally faced a seed shortage. Dogwood seed crop is affected by several factors. A year of heavy flowering and seed load is often followed by reduced flowering, and thus less seed, the following year (15). Cold winters and late spring freezes can damage flowers and reduce fruit formation (3). While winter damage to dogwood flowers usually only occurs in the northern limits of its adaptation, spring freeze damage can occur throughout much of the flowering dogwood growing area. Recent powdery mildew (*Erysiphe pulchra*) problems on flowering dogwood have exacerbated the seed supply situation, as heavy infestations of powdery mildew have resulted in reduced flower bud formation (4).

Storing dogwood seed during years in which an abundance of seed is available for use when seed are scarce would help growers stabilize their seed supply. It has been reported (7) that air-dried dogwood seed can be stored for 4 to 8 years at 3 to 5C (38 to 41F), but seed moisture content of the stored seed was not specified nor were other storage temperatures

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tested. The objective of this study was to investigate the effects of seed moisture content and storage temperature on dogwood seed viability.

Materials and Methods

Seed collection. Dogwood berries were collected in Fall 1999, 2000 and 2002 from flowering dogwood plants growing at the Tennessee State University Nursery Research Center in McMinnville, TN. The berries were obtained from the cultivars 'Cherokee Brave', 'Cherokee Princess', 'Cherokee Chief', 'Barton', and 'Cloud 9' growing in a landscape setting and from seed-derived plants growing in a native stand. Each year, berries from all sources were bulked after collection.

The flesh of the fruit was removed using a commercial seed cleaner (Bouldin and Lawson, McMinnville, TN) Cleaned seed were placed in a tub of water, and any seed that floated to the top discarded. After the surface of the remaining seed had dried, seed were placed into zip-top plastic bags and stored at 5C (41F) for up to 1 week.

Germination of control. Three 50-seed samples from the 1999 and 2000 seed lots were placed into moist vermiculite in zip-top plastic bags within 1 week of collection and stored at 5C (41F) for 15 weeks. Seed then were sown in 12.7 cm (5 in) square pots (25 seed/pot) containing aged pine bark amended with 6.5 $kg \cdot m^{-3}$ (11.0 $lb \cdot yd^{-3}$) 19N–2.1P–7.4K Osmocote fertilizer (Scotts-Sierra Horticultural Products Co., Maryville, OH), 0.6 $kg \cdot m^{-3}$ (1.0 $lb \cdot yd^{-3}$) Micromax (Scotts-Sierra Horticultural Products Co.), and 0.2 $kg \cdot m^{-3}$ (0.4 $lb \cdot yd^{-3}$) Epsom salts. Pots were placed in a heated greenhouse under natural light conditions and over-head watered as necessary. Maximum and minimum air temperatures during germination of the control seed samples were 26.7/15.6C (80/60F) for the 1999 seed lot and 25/16.6C (77/62F) for the 2000 seed lot. After 4 weeks, number of seedlings was counted. In those cases in which a stone contained two seeds and both germinated, only one seedling was included in the count. Percent germination of the three samples was averaged, and the mean served as the control germination percentage.

Seed storage. The effect of seed storage on germination was tested using seed collected in 1999 and 2000. Three 50-seed samples were used for determining initial seed moisture content (MC). Seed were weighed and then dried in a 105C (221F) oven for 24 hours. Each sample of seed was weighed again and MC determined using the following formula: (fresh weight – dry weight) / fresh weight = MC. Mean MC of the three samples was used as the initial MC for the seed collection.

The remaining seed were divided into three equal batches and each batch was weighed. A target seed weight was calculated for each batch using the following formula: $x - ax = b(1 - c)$, where x = target weight, a = desired MC, b = initial weight, and c = initial MC. Target weights for 6, 10 and 14% MC were determined.

Seed were spread in a single layer on paper towels in a 22C (72F) laboratory. Each batch of seed was weighed at least hourly until it reached the target weight. In order to reach 6% MC, it was necessary to place an incandescent 60W light approximately 18 inches above the seed during the final 2 to 3 hours of drying. Once seed had reached the desired MC it was either immediately packed for storage or placed in a zip-top plastic bag and stored at 5C (41F) overnight. Seed that were stored overnight were re-weighed the following morning to ensure that MC had not changed, and then were immediately packaged for storage.

Seed were placed in 20-ml glass bottles fitted with a screw-top lid for storage, with each bottle containing 50 seed. For each MC, seed were stored at 22C (72F), 5C (41F), and –20C (–4F). A household refrigerator/freezer was used for storing the 5C and –20C samples, while the 22C seed were stored in the laboratory. All seed were stored in the dark. Seed were stored for 1, 2 and 3 years.

Germination of stored seed. At the end of each storage period, seed were stratified and planted in a greenhouse as described above for the control. Maximum and minimum air temperatures during germination of the stored seed were 25/16.6C (77/62F) in 2001, 27.9/15.5C (82/60F) in 2002, 25.6/16.9C (78/63F) in 2003, and 25.7/15.5C (78/60F) in 2004. Seed germination, which was defined as the presence of above-ground tissue, was determined after 4 weeks. Germination data for the stored seed was converted to a percentage of the control seed germination. The above-ground portion of the seedlings was harvested, dried at 60C (140F) for 48 hr, and dry weight determined. In cases where two seedlings germinated from a stone, only the larger seedling was harvested and dried. Dry weight for each treatment was divided by the number of seed that germinated to give a mean dry weight/seedling value.

Changes in MC during storage. Seed collected in Fall 2002 were used to determine if changes in seed MC occurred during storage. Seed were dried to 6, 10 and 14% MC and prepared for storage as described for the 1999 and 2000 seed lots. Prior to storage at 22, 5 and –20C (72, 41 and –4F), the weight of each 50-seed sample was determined. Seed were stored for 1 week, 4 weeks, 3 months, 6 months and 1 year and then re-weighed. Separate samples were used at each of the sampling dates. MC of the stored seed was determined using the following formula: $x = (ab - a + c) / c$; where x = MC of stored seed, a = weight of fresh seed, b = MC of seed prior to storage, c = weight of stored seed.

Statistical analysis. Data from 1999 and 2000 seed lots and for different lengths of storage were analyzed separately. Each experiment consisted of nine treatments, representing all seed MC × storage temperature combinations, and was replicated three times. When germination of a treatment dropped to 0%, that treatment was removed from the following year's statistical analysis. No transformation of germination percentages was required since the equal variance assumption was not violated. Germination and seedling weight data were analyzed using the General Linear Model Procedure of SigmaStat (SPSS Inc., Chicago). Where data were significant, Fishers' LSD procedure was used to separate means.

Results and Discussion

1999 seed lot. The control seed samples from the 1999 seed lot had a mean germination rate of 58.7% ± 1.76. Initial MC of the 1999 seed lot was 20.9% ± 0.03.

After 1 year in storage at 22C (72F), seed dried to 10 and 14% MC failed to germinate and seed dried to 6% MC had

Table 1. Effect of storage temperature and seed moisture on viability of flowering dogwood seed.

Storage temperature	Seed moisture	1 year storage		2 years storage		3 years storage	
		Germination, % of control[zy]	Dry weight/ seedling (mg)	Germination, % of control	Dry weight/ seedling (mg)	Germination, % of control	Dry weight/ seedling (mg)
Seed collected Fall 1999							
22C	6%	28.9c	13.8d	0.0b	—	—	—
	10%	0.0d	—	—	—	—	—
	14%	0.0d	—	—	—	—	—
5C	6%	53.3b	27.6b	74.4a	29.3a	72.2bc	34.0a
	10%	66.7b	30.9a	86.7a	17.6b	63.3c	12.5b
	14%	55.6b	21.0c	8.9b	4.9c	0.0d	—
–20C	6%	70.0b	28.5ab	80.0a	24.2ab	108.9a	38.7a
	10%	72.2b	28.1b	88.9a	30.8a	99.4ab	31.9a
	14%	94.4a	28.4ab	94.4a	25.7ab	76.3bc	32.3a
Seed collected Fall 2000							
22C	6%	3.3e	7.8d	0.0c	—	—	—
	10%	0.0e	—	—	—	—	—
	14%	0.0e	—	—	—	—	—
5C	6%	42.5b	16.0bc	48.8b	10.8d	13.4c	8.7c
	10%	26.7c	18.5bc	96.7a	25.9c	69.2b	17.5b
	14%	15.8d	13.2cd	50.8b	11.5d	0.0d	—
–20C	6%	48.3b	21.3ab	106.7a	32.4a	91.7a	21.9a
	10%	44.2b	20.5ab	107.5a	29.1b	87.2a	21.3a
	14%	62.5a	26.2a	100.8a	31.5ab	84.8a	20.9a

[z]Values within a column and a seed collection followed by the same letter do not differ significantly according to Fisher's LSD test ($P \leq 0.05$). n = 3.
[y]Germination of control: 1999 seed lot, 58.7%; 2000 seed lot, 80.0%.

poor germination and low seedling dry weight (Table 1). Germination of seed stored at 5 and –20C (41 and –4F) ranged from 53.3 to 94.4% of the control, with seed dried to 14% MC and stored at –20C (–4F) having the highest germination rate and one of the highest seedling dry weights.

After 2 years in storage, all seed stored at 22C (72F) failed to germinate. With the exception of seed dried to 14% MC and stored at 5C (41F), which had very poor germination and low seedling dry weight, all treatments stored at 5 or –20C (41 or –4F) for 2 years had germination rates of at least 74% of the control.

Seed dried to 14% MC and stored at 5C (41F) failed to germinate after 3 years in storage. Seed dried to 6% MC and stored at –20C (–4F) germinated better than all other treatments, with the exception of that dried to 10% MC and stored at –20C (–4F). After 3 years in storage, all seedlings had similar weights except for the treatment in which seed had been dried to 10% MC and stored at 5C (41F).

2000 seed lot. The control seed samples for the 2000 seed lot had a mean germination rate of 80% ± 1.15. Initial MC of the seed collected in 2000 was 20.3% ± 0.06.

As in 1999, after one year in storage at 22C (72F), seed dried to 10 and 14% MC failed to germinate and seed dried to 6% moisture germinated poorly (Table 1). Seed germination and seedling dry weight were highest for seed dried to 14% MC and stored at –20C (–4F).

None of the seed stored at 22C (72F) germinated after 2 years in storage. All seed samples stored at –20C (–4F) and the seed dried to 10% MC and stored at 5C (41F) had germination rates similar to the control. Seedling weight was highest for samples stored at –20C (–4F).

After 3 years in storage, seed stored at –20C (–4F) had the highest germination and seedling dry weight. Of the samples stored at 5C (41F), those dried to 14% MC failed to germinate and those dried to 6% had very low germination and seedling dry weight.

Change in MC during storage. Only minor changes in seed weight were observed in seeds stored at any of the storage periods. Because of the similarity in results from all sampling periods, data are presented only for the shortest and longest storage periods (Table 2). While the weight of the seed dried to 6 and 10% MC remained almost constant throughout the storage period, seed dried to 14% lost up to 0.6% MC.

Two categories of seed storage behavior have been defined (8). Orthodox seeds can be safely dried to a low MC without damage to the seed. The longevity of stored orthodox seeds generally increases with decreases in seed MC and storage temperatures (6). In contrast, recalcitrant seeds quickly lose viability when dried or frozen. A previous study

Table 2. Changes in flowering dogwood seed moisture content during storage.

Storage temperature	Seed moisture(%) prior to storage	Seed moisture (%)[z] after storage for:	
		1 week	1 year
22C	6	6.0 ± 0.01	6.1 ± 0.02
	10	9.9 ± 0.02	10.0 ± 0.01
	14	13.6 ± 0.13	13.4 ± 0.09
5C	6	6.0 ± 0.00	6.0 ± 0.01
	10	10.0 ± 0.01	10.0 ± 0.02
	14	13.6 ± 0.14	13.8 ± 0.15
–20C	6	6.1 ± 0.02	6.0 ± 0.01
	10	10.0 ± 0.01	10.0 ± 0.02
	14	14.0 ± 0.04	13.8 ± 0.07

[z]Mean ± s.e.; n = 3.

indicated that dogwood seed exhibit orthodox storage behavior (7), an observation that was confirmed by the results of this study.

Both storage temperature and seed MC affected dogwood seed viability. Seed germination and seedling dry weight dropped rapidly when flowering dogwood seed were stored at room temperature. Seed stored in a 5C (41F) refrigerator maintained better viability than those stored at 22C (72F), especially if dried to 6 to 10% MC prior to storage. However, the highest germination percentage and seedling weight were obtained from those seed that were stored at –20C (–4F). After 1 year in storage at –20C (–4F), the best germination was obtained from seed stored at 14% MC, but all MCs produced similar results after 2 years in storage. While results from the 1999 and 2000 seed lots differed somewhat on the effect of MC on germination after 3 years in storage at –20C (–4F), the seed dried to 6% MC seemed to germinate as well, if not better, than those dried to 14% MC. MC may become more critical during storage at –20C (–4F) if seed are stored for longer than 3 years.

At each MC, germination rates of the seed collected in 2000 and stored at 5 and –20C (41 and –4F) for 1 year were approximately half that of those stored for 2 years. Seed collected in 2000 and stored 1 year were germinated in Spring 2002 under the same conditions as the seed collected in 1999 and stored 2 years. Since germination of the 1999 seed lot that had been stored for 2 years at –20C (–4F) ranged from 80 to 94% of the control, it does not appear that the low germination rate of the 2000 seed in 2002 was due to poor environmental conditions during germination. No clear-cut explanation for the poor germination of the 2000 seed lot following 1 year in storage can be given.

Because seed are hygroscopic, it is important to regulate the relative humidity of atmosphere under which they are stored. In this study, seed were stored in sealed glass bottles and MC remained fairly constant during storage. Since the seed only occupied about one-third of the volume of the storage bottle, the small loss in MC that occurred in the seed that had been dried to 14% MC was probably due to seed moisture reaching equilibrium with the ambient relative humidity of the bottle.

Information has been provided to producers that air-dried dogwoods seed will maintain good viability for 4 to 8 years if the seed are stored at 3–5C (38–41F) (1, 2, 7). Our results indicate that dogwood seed retain better viability if stored at –20C (–4F) than at 5C (41F). If seed are to be stored at 5C (41F), it is critical than they be dried to 6 to 10% MC prior to storage. Drying dogwood seed below 6% MC is not recommended as seed dried to very low MC often germinates very poorly (12). Time required to dry seed to the desired moisture content depends on initial seed moisture content, air temperature, relative humidity and thus will vary between years and locations. Determining the MC of a small sample of seed periodically during the drying process will ensure that seed are dried to optimum MC.

Based on the results of this study, it is recommended that dogwood seed be dried to 6 to 10% MC prior to storage and stored in a –20C (–4F) freezer in air-tight containers. Use of these guidelines should allow growers to store flowering dogwood seed for at least 3 years without a significant loss in viability.

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