Causes and Breaking of Seed Dormancy in Flowering Dogwood (*Cornus florida* L.)

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Abstract. *Cornus florida* seeds show strong dormancy. In this study, we investigated the causes of the dormancy by assessing the permeability of the stony endocarp, the germination of seeds after mechanical dissection, and the effect of endogenous inhibitors. Water uptake by intact and cracked seeds during imbibition showed that the endocarp formed a strong barrier for water absorption. Meanwhile, extracts from endocarp decreased the germination frequency of chinese cabbage seeds from 99.3% (control) to 2.7%. Therefore, the endocarp was the mechanical barrier and contained endogenous inhibitors for seed germination. However, the germination percentage of decoated seeds and dissected seeds with the exposed radicle were only 13.3% and 28.7%, respectively. It was found that the endosperm also played a role in seed dormancy. Extracts from endosperm decreased the germination frequency of chinese cabbage seeds from 99.3% (control) to 53.0%. By contrast, extracts from embryo did not affect the germination of chinese cabbage seeds. When tested with the excised embryos, germination percentage was up to 85.3% at the 16th day of incubation. Taking these results together, we concluded that the endocarp and endosperm were responsible for seed dormancy in *C. florida*. To break the seed dormancy of *C. florida*, stratification and soaking in sulfuric acid are the effective means. The highest germination frequency was achieved by immersing seeds in 98% sulfuric acid for 10 minutes, then soaking the seeds in 500 mg·L⁻¹ gibberellic acid (GA₃) for 72 hours before cold stratification at 5 °C for 60 days.

Flowering dogwood (*C. florida* L.), a member of the subgenus *Benthamidia* in *Cornus*, is a small- to medium-sized deciduous tree native to eastern North America (Borer et al., 2013; McLemore, 1990; Rushforth, 1999). The species is prized for the ornamental value of the pink, red, or white petal like, enlarged bracts in early spring, and brilliant red foliage and bright red berries in autumn. As noted by the U.S. Department of Agriculture Census of Horticultural Specialties (USDA-NASS, 2010) from retail and wholesale sales, in the United States, and especially in states such as Tennessee, *C. florida* is an economically important ornamental tree and may also be used as an important food source for wildlife in forests when its fruits ripen in autumn (Paul and David, 2008; Stiles, 1980). In addition, *C. florida* is widely considered as a calcium pump, increasing the rates of mineralization of the forest litter and soil because of the high calcium concentrations in

its foliage (Holzmueller et al., 2007; Thomas, 1969).

Typically, *C. florida* is propagated from seeds, but the seeds usually develop strong dormancy (Coartney et al., 1989), which may be a great challenge for seed reproduction (Dirr and Heuser, 1987). Studied with *C. kousa* var. *Chinensis*, Fu et al. (2013) indicated that the dormancy of chinese dogwood could be classified as physiological dormancy due to the existence of inhibitory substances in the endosperm. However, the causes of *C. florida* seed dormancy remains largely unknown thus far, except that Laufmann and Wiesner (1998) reported rapid germination of eastern dogwood (*C. florida* cv. Small) by embryo extraction, cut cotyledons, and GA₃. Normal seed germination of *C. florida* requires a moist prechill treatment at 3–5 °C for 3–4 months. In this study, we aimed to identify the multifaceted causes and to establish a practical and powerful method to successfully break the seed dormancy of *C. florida* that can be applied in seed reproduction practice.

Materials and Methods

Source of seeds. The seed of *C. florida* is encased within a stony endocarpic pit (hereafter seeds with endocarp are referred to as the seed). In this study, the seeds of *C. florida* were imported via Lovelace Seed Company (Elsberry, MO) in Spring 2013 and stored at 4 °C. The seeds were collected from Knoxville, TN.

Water permeability of the endocarp. To test whether the hard stony endocarp may restrict water imbibition for germination, two experiments were performed to test the water absorption capability of the seed. In Expt. I, 30 intact seeds enclosed within the endocarp were weighed on an electronic balance accurate to 0.001 g, then were immersed in 200 mL distilled water at room temperature for 168 h. At 12-hour intervals, the seeds were removed from the water, surface dried using filter paper, and weighed. The seeds were then reimmersed. The final weight measurement was recorded after soaking for 14 cycles for 168 h. In Expt. II, the same procedure was repeated except for the fact that the endocarp was cracked in the micropylar region of pliers, otherwise, the procedures were identical to those used in Expt. I. The two experiments were conducted using three replicates of 30 seeds each. In each experiment, the water uptake ratio was calculated and expressed as a percentage of the seed fresh weight.

Germination after mechanical manipulation.

Three experiments were performed to identify whether different tissues restrict the germination of *C. florida* seeds. Before the experiments, seeds were treated as follows: a) the stony endocarp was completely removed following breakage using a pair of pliers (“decoated seeds”) (Fig. 1A); b) the decoated seeds were carefully dissected using a single-side blade to expose the radicle (“radicle-exposed seeds”) (Fig. 1B); and c) the embryo was excised from the seed (Fig. 1C). After these preliminary treatments, the materials were incubated on moist absorbent cotton at 25 °C and an 8-h photoperiod. During incubation, the seeds were watered every 2 d. Germination was monitored every second d for 16 d. The germination percentage was determined in accordance with the International Seed Testing Association (ISTA) (2011) guidelines. Three replicates of 50 seeds were used in each of the three experiments.

Extraction of inhibitors of seeds. Inhibitors of *C. florida* seed germination were extracted from the endocarpic pit, the endosperm, and the embryo using methanol as the extract solvent. The extraction procedure was conducted as that described by Hou et al. (2014). First, the endocarps, the endosperms, and the embryos were excised separately. Then, the endocarps were ground into powder in a mill grinder, and the endosperms and the embryos were separately ground into powder in liquid nitrogen. The powdered samples were extracted with methanol in a soxhlet apparatus. The extracts were obtained by the following procedures: the powdered samples (2.5 g) were suspended separately in 40 mL of 80% (v/v) methanol at 5 °C for 24 h three times. Subsequently, the combined supernatants from the extractions for each tissue were evaporated under vacuum at 37 °C to remove the solvent completely. Finally, 2 mL of each extract was diluted to 100 mL with distilled water.

The activity of inhibitors in the extracts was determined by incubating nondormant chinese cabbage seeds in petri dishes moistened with the extracts. Three replicates of

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Fig. 1. Germination of *C. florida* seeds after mechanical dissection. (A) The decoated seeds with intact endosperm and embryo; (B) the decoated seeds with radicles exposed; (C) the excised embryos; (D) germination of excised embryos after 7 d.

100 cabbage seeds were used for each extract. Seeds in each replicate petri dish were soaked in 5 mL of the extract solution for 3 h, whereas control seeds were soaked in distilled water for the same period. The seeds were incubated in a growth chamber at 25 °C with an 8-h/16-h (light/dark) photoperiod. Seedling emergence was counted daily for 6 d. A seedling was classified as emergent in accordance with the ISTA (2011) rules. Germination percentage was calculated based on the number of normal seedlings that emerged as a proportion of the total number of seeds sown.

Evaluation of methods to break seed dormancy. We applied the following treatments to evaluate their capability to break the dormancy of *C. florida* seeds. In treatment 1, seeds were immersed in 500 mg·L⁻¹ GA₃ for 48, 60, 72, and 84 h at room temperature. A parallel control was conducted using seeds imbibed in water for 84 h without GA₃. The water or GA₃ solution was changed twice daily (Hartmann et al., 1989). After soaking, the seeds were stratified at 5 °C (cold stratification) for 90 d. In treatment 2, seeds were soaked as in treatment 1, but were stratified during the d at 15 °C and during the night at 5 °C (alternative stratification) for 90 d. During stratification, water was added to keep the sand with water-holding capacity of 50% to 60%. In treatment 3, seeds were soaked in concentrated sulfuric acid (98%) with seeds: acid of 1:2 (v/v) for 0, 5, 10, or 20 min (chemical scarification). During treatment, the solutions were stirred continuously with a glass rod. After chemical scarification, the seeds were washed in running water for 24 h. Next, the seeds were soaked in 500 mg·L⁻¹ GA₃ for 72 h and stratified at 5 °C for 60 d.

After completion of the stratification treatment, the germinability of four replicates of

50 seeds each was tested in moistened sand at 25 °C with an 8-h/16-h (light/dark) photoperiod. The number of germinated seeds was counted every other day for 30 d. During the germination test, water was added to keep the seeds moist. A seedling was classified as emergent in accordance with the ISTA (2011) rules. The germination percentage was calculated as described above.

Statistical analysis. A completely randomized design was used in all experiments. The effect of the treatments was assessed by the final germination percentage for each trial. The significance of differences between the means was assessed using the least significant difference (LSD) test with SPSS v19.0 (<http://support.spss.com/>).

Results

Water absorption by *C. florida* seeds. The cracked seeds absorb water more rapidly than the intact seeds. The water uptake by cracked seeds increased to a saturation level of 16.9% in 24 h, and then leveled off. In contrast, the water uptake by intact seeds only increased to 7.3% in 24 h, and it would take at least 168 h for the intact seeds to achieve a nearly saturation level of water uptake (Fig. 2).

Germination after mechanical manipulation of seeds. The excised embryos showed no dormancy. After incubation for 7 d, the embryonic axis elongated and the cotyledons of excised embryos started to expand and turned green (Fig. 1D). The germination percentage was 85.3% after 16 d of incubation. In contrast, the germination percentage of decoated and radicle-exposed seeds were only 13.3% and 28.8%, respectively, after 16 d of incubation.

Inhibitor activity of extracts from *C. florida* seeds. Extracts from the excised

embryos of *C. florida* showed no significant effect on cabbage seed germination ($P = 0.217$) comparing to the control, whereas extracts from the endosperm significantly ($P < 0.001$) reduced the percentage germination of cabbage seeds from 99.3% (control) to 53.0%. Soaking cabbage seeds in the same concentration of extracts from the endocarp had an even more significant ($P < 0.001$) negative effect on cabbage seed germination (reduced to 2.7%). It indicates that both the endosperm and the endocarp contain endogenous inhibitors, which play an important role in the dormancy of *C. florida* seeds.

Effects of cold and chemical scarification on *C. florida* seed germination. Germination responses under different GA₃ soaking periods combined with stratification treatments showed significant differences. Generally, with increasing duration of soaking in GA₃ up to 72 h, seed germination increased significantly ($P < 0.05$) in both treatment 1 and treatment 2. Compared with the control (soaking in GA₃ for 0 h combined with 90 d of cold stratification), for which 22.3% germination was recorded, seed germination increased to 79.0% when seeds were soaked in GA₃ for 72 h followed by 90 d of cold stratification (Table 1). For the same duration of soaking in GA₃, germination in treatment 1 (cold stratification) was slightly higher than that in treatment 2 (alternative stratification), except for soaking in GA₃ for 0 and 60 h (Table 1). Statistical analysis showed that the effect on seed germination of cold stratification was not significantly different from that of alternative stratification ($P > 0.05$). However, we observed that the germination speed was more erratic when seeds were treated with alternative stratification. Therefore, seeds were only cold stratified at 5 °C (cold stratification) in treatment 3. It is also notable that soaking seeds in GA₃ for 72 h had the best effect on seeds germination in both treatment 1 and treatment 2 (Table 1), thus only 72 h-GA₃ soaking was performed in treatment 3.

To shorten the duration of cold stratification, seeds were scarified in sulfuric acid before GA₃ soaking (72 h) in treatment 3. With chemical scarification, most seeds germinated at cold stratification of 60 d, and we achieved seed germination percentage of 81.8% at cold stratification of 60 d when seeds were first scarified in sulfuric acid for 10 min (Table 1). Thus, chemical scarification shorten the duration of cold stratification from 90 to 60 d. However, if we increased the scarification duration to 20 min, seed germination percentage would be greatly reduced (lowered to 5.5%). This indicated that excessive chemical scarification would cause damage to the seeds.

Discussion

Causes of *C. florida* seed dormancy. Water uptake is a fundamental requirement for the completion of seed germination (Manz et al., 2005). In this study, water absorption by intact seeds increased slowly

Fig. 2. Water absorption curves of the intact and cracked seeds of *C. florida*.

Table 1. Effects of different treatments on *C. florida* seed germination.

Treatment	Duration in sulfuric acid (min)	Stratification	Germination percentage of different GA ₃ soaking time treatments (%)				
			0 h	48 h	60 h	72 h	80 h
Treatment 1	0	Cold stratification for 90 d	22.3 ± 0.3 a	39.7 ± 0.9 b	50.3 ± 1.5 c	79.0 ± 1.5 e	74.7 ± 1.2 d
Treatment 2	0	Alternative stratification for 90 d	25.5 ± 0.5 a	28.3 ± 1.2 a	51.5 ± 1.2 b	74.8 ± 0.9 d	56.3 ± 0.9 c
Treatment 3	0	Cold stratification for 60 d	—	—	—	63.0 ± 2.0 c	—
	5		—	—	—	70.5 ± 1.3 b	—
	10		—	—	—	81.8 ± 2.0 a	—
	20		—	—	—	5.5 ± 1.3 d	—

Values of germination percentage are displayed with the mean ± SE ($n = 50$). In treatment 1 and treatment 2, germination percentages followed by the same letters in the same row indicated no significant difference at $P \leq 0.05$ by the LSD test, whereas those followed by different letters in the same row indicated significant difference at $P \leq 0.05$ by the LSD test. Both treatment 1 and treatment 2 indicated that soaking seeds in GA₃ for 72 h had the best effect on seed germination, thus only 72 h-GA₃ soaking was performed in treatment 3 (“—” indicates germination is not carried out at the corresponding period of GA₃ treatments). In treatment 3, significance test was made for germination percentages of seeds soaking in sulfuric acid for different duration.

to 15.8% after imbibition for 168 h (Fig. 2). This finding indicated that the endocarp of *C. florida* could restrict water uptake and that might be one cause of seed dormancy. However, water uptake by seeds with a cracked endocarp was 16.9%, only marginally higher than that of intact seeds, which was even lower than that of intact seeds from *C. kousa* var. *chinensis* (Fu et al., 2013). Thus the water content was likely to be inadequate for the metabolic processes required for germination. Therefore, in addition to the endocarp, the endosperm may also retard water absorption. The identity of factors that influence water uptake and the water absorption mechanism in *C. florida* requires further exploration.

Laufmann and Wiesner (1998) reported that the cotyledons of eastern dogwood (*C. florida* L. cv. Small) significantly inhibited germination of excised embryos. In this study, the germination percentage of excised embryos with cotyledons was 85.3%, and extracts from excised embryos did not inhibit the germination of cabbage seeds. Thus, the cotyledons did not inhibit the germination of excised embryos of *C. florida* seeds. Fu et al. (2013) also found that there was no dormancy in the excised embryos with cotyledons of *C. kousa* var. *chinensis*. We proposed that the effect of cotyledon on seed dormancy might vary among different *Cornus* species. Germination of decoated and radicle-exposed seeds was significantly lower than that of

excised embryos, which indicated that the endosperm was a barrier to seed germination. Extracts from the endosperm and endocarp significantly decreased cabbage seed germination, which suggested that an endogenous inhibitor might contribute to *C. florida* seed dormancy. Our findings indicate that seeds of *C. florida* show both physical and physiological dormancy as the restriction of water absorption and the inhibition of extracts, which belongs to the combinational dormancy category according to the seed dormancy classification of Baskin and Baskin (2004).

Methods of breaking *C. florida* seed dormancy. Many procedures, such as stratification with low or alternate temperatures (Ertekin, 2010; Li et al., 2013; Murat et al., 2010), plant hormones, and other chemical treatments (Li et al., 2012; Mark, 1994) are effective in overcoming seed dormancy in a wide variety of species (Bewley and Black, 1982; Cohn, 1987, 1990; Laufmann and Wiesner, 1998). To break the physical dormancy, sulfuric acid is the effective scarifying agent (Bhatt et al., 2000). The data reported herein showed that soaking seeds with 500 mg·L⁻¹ GA₃ for 72 h combined with cold stratification was effective to break seed dormancy of *C. florida* (Table 1), while it required a long period of cold stratification (90 d). Soaking seed in sulfuric acid (98%) for 10 min before GA₃ treatment would reduce cold stratification requirement to

60 d. Immersion of *C. florida* seeds in sulfuric acid scarified the hard endocarp, which would accelerate water absorption and gas exchange, and ultimately results in rapid and uniform germination (Everitt, 1983; Hartmann et al., 1989). However, soaking the seeds in sulfuric acid for longer time would drastically decrease the germination percentage (Table 1), indicating excessive scarification would damage the seeds. Based on our study, rapid promotion of *C. florida* seed germination was achieved by immersing seeds in sulfuric acid for 10 min before soaking in 500 mg·L⁻¹ GA₃ for 72 h followed by cold stratification at 5 °C for 60 d. As a conclusion, we have established an appropriate method to break *C. florida* seed dormancy that can be applied in practical seed reproduction practice.

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