Germination of Fully Developed *Corylopsis* Seeds Influenced by Harvest Date and Cold Stratification

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Abstract. *Corylopsis* seed germination tests were conducted to assess the influence of harvest date (seed maturity) and cold stratification (CS) at 5 °C. *Corylopsis gotoana* seeds harvested on 12 July, 2 and 22 Aug., 6 and 20 Sept., and 1 and 10 Oct. 2011 were immersed in water for 20 min to separate fully developed seeds (full seeds) from empty seeds by floatation, and by X-ray scanning to identify full from empty seeds (Expt. 1). Immersing seeds in water did not effectively separate full seeds from empty seeds as evaluated by seed germination tests. Seeds harvested on or around 6 Sept. that sank showed translucent X-ray images with fully developed internal structures composed of embryo, cotyledons, and endosperm, and were considered mature. Without CS, >12% seeds harvested on 20 Sept. germinated, regardless of whether seeds were full or empty. Seeds of *C. coreana* harvested on 5 and 15 Sept., and 5 and 18 Oct. were stored dry at 20 °C until 27 Dec. and germinated after 0, 3, 6, 9, and 12 weeks of CS (Expt. 2). Longer than 6 weeks of CS was required to accelerate and increase the germination of seeds harvested on or after 5 Sept. Germination percentage of full seeds harvested on Oct. 18 was increased to >72% as the duration of CS treatment increased to 12 weeks. In conclusion, fully developed seeds harvested on or after 6 Sept. were considered mature and 6 weeks of CS accelerated germination and increased the germination percentage. Further, dormancy of *Corylopsis* seeds appears to be shallow since germination occurred without any CS.

Corylopsis Siebold & Zucc. is a genus of about 30 species in the Hamamelidaceae family, comprised of shrubs native to eastern Asia cultivated for very early spring blooms and attractive autumn foliage (Roh et al., 2007). Germination of seeds in many woody plants, including *Corylopsis*, requires a period of warm stratification (WS) at 20 °C followed by cold stratification (CS) at 5 °C to break dormancy (Baskin et al., 2002; Batlla and Benech-Arnold, 2003; Dirr, 1990). The principal characteristics of dormancy have been

thoroughly reviewed (Baskin and Baskin, 2004; Bewley, 1997; Finch-Savage and Leubner-Metzger, 2006; Hilhost, 2007). However, gaps still remain in our understanding of dormancy involving various taxa, including *Corylopsis*.

Mature *Corylopsis* seeds showed fully developed internal structure of vegetative organs when examined by X-ray imaging (Kim et al., 2016, 2017). Although dry seeds stored at 5 °C for two years germinated (J.H. Kim, unpublished data), no clear or definitive documentation of desiccation tolerance in *Corylopsis* seeds is available. Therefore, the nature of dormancy in *Corylopsis* seeds is not well understood: if seeds exhibit deep or nondeep physiological dormancy, morphological dormancy with underdeveloped embryo, or physical dormancy imposed by the seedcoat. In a few woody plant species with physiological and/or morphological

dormancy, seed dormancy can be released, for example, by WS at 20 °C and by CS at 5 °C. To maximize the germination it is necessary to understand the upper limit of CS and/or lower limit of WS.

Corylopsis glabrescens seeds were described as requiring 3 to 5 months of WS followed by 3 months of CS before germination (Dirr, 1990); however, *C. coreana* and *C. sinensis* var. *calvescens* seeds germinated readily at more than 90% after 1 month of WS and 2 months of CS and a few seeds germinated at 10 °C without cold treatment (Roh et al., 2008). When freshly harvested seeds of *C. coreana* were subjected to WS at either 15–25 °C or 18.5/18 °C (day/night) without subsequent CS, germination rate was very low (Roh et al., 2004). Thus, differences in the germination requirements of different species of *Corylopsis* appear to exist. Other studies (Kim et al., 2016) suggest that chemical inhibitors may also play a role in the dormancy of *Corylopsis* seeds as indicated by the increase in germination of *C. sinensis* var. *calvescens* seeds after immersion in water for 16 h or after treatment with ethanol.

There are no reports on the time of harvesting mature *Corylopsis* seeds to ensure maximum germination. If the embryo was not mature when fruits were harvested early, seeds will not germinate, which has never received adequate attention. In *Styrax japonicus* Siebold & Zucc., immature seeds did not show well-developed embryo and cotyledons when evaluated by magnetic resonance imaging (MRI) and it was possible to achieve >80% germination in mature seeds harvested 12–16 weeks after anthesis (Roh et al., 2004).

To maximize germination percentages of *Corylopsis* seeds, empty seeds that float when immersed in water should be separated from fully developed and mature seeds that sink, followed by optimum CS treatment. Immersing seeds of *C. sinensis* var. *calvescens* in water for 16 h increased the number of full seeds that germinated without CS (Kim et al., 2017), suggesting that physiological dormancy is not deep and dormancy inducing substance(s) present in the seedcoat may have been leached out. X-ray images of *C. coreana* seeds were used to separate fully developed mature seeds with developed internal structure from empty seeds, and many seeds can be imaged simultaneously by X-ray imaging to select full seeds on a large scale in *Corylopsis* (Kim et al., 2017). It was also possible to understand the internal structures generating full and well-developed seeds with translucent images of castor bean (*Ricinus communis* L.) seeds to improve the seed lot quality and germination (Carvalho et al., 2010).

Information on germination of *Corylopsis* seeds as influenced by seed maturity and CS at 5 °C is not readily available. Therefore, these experiments were designed a) to separate fully developed seeds from empty seeds by X-ray imaging and b) to investigate the seed germination of *C. coreana* and

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C. gotoana influenced by seed harvest dates (maturity) and with CS.

Materials and Methods

Plant materials. Seeds were harvested from *Corylopsis coreana* Uyeke from a population of stock plants grown from seeds initially collected from Namhae, Gyongsangnamdo, Korea, and from *C. gotoana* Makino collected from Kyushu, Japan. Seeds of both species were packed in a moist Pro-Mix BX medium (50% moisture) and received 5 °C treatment (CS) and germinated as described by Kim et al., (2016). Seeds were sown in a 15-cm pot filled with a Pro-Mix BX (Premier Horticulture Inc., Quakertown, PA) growing medium and received temperature treatments as specified in each experiment. When hypocotyls emerged from the sowing medium, seeds were recorded as germinated. Each treatment was replicated two or three times, with the number of seeds per replication specified in each experiment.

Germination of *C. gotoana* seeds influenced by seed harvest dates and X-ray images (Expt. 1). *Corylopsis gotoana* fruits harvested on 12 July, 2 and 22 Aug., 6 and 20 Sept., and 1 and 10 Oct. 2011 were kept at 20 °C for 3 to 4 d and seeds were separated from the fruits. Weights of 10 individual seeds that either sank or floated after immersing in water for 20 min, except for seeds harvested on 12 July, were recorded. Seeds that were harvested from 20 Sept. onward were easily separated from the fruits; the fruits turned brown and started to split open showing seeds inside.

Seeds that sank or floated were air-dried at 20 °C, stored dry at 5 °C, and sown on 17 Nov. and germinated in a growth chamber as described by Kim et al. (2017). The number of seeds per replication harvested on 12 July and 2 Aug. was 15 and 39 seeds, respectively, while seeds harvested on 22 Aug. or later contained 100 seeds per replication; there were three replications per treatment (Table 1). Germination of X-ray imaged seeds following exposure to 20kV for 15 s was as described previously (Kim et al., 2017). After 30 consecutive days without additional germination, pots were moved on

29 Jan. 2012 for CS for 3 months, and returned to the growth chamber, where germination was recorded daily.

Only the data for seeds germinated by the first week after planting and the final germination percentages are presented. Regression analyses were performed following arcsine transformation (Statistical Analysis System, 2002). The means were transformed to the original data and presented. The number of days to the final germination and the germination percentages were presented.

Germination of *C. coreana* seeds influenced by seed harvest dates and duration of cold treatment (Expt. 2). *Corylopsis coreana* seeds harvested on 5 and 15 Sept. and 5 and 18 Oct. 2011 were stored dry at 20 °C until 27 Dec. 2011. Only seeds that sank after immersing in water for 15 min were packed in moist Pro-Mix BX and treated for 0, 3, 6, 9, and 12 weeks of CS. Another group of *C. coreana* seeds harvested on 18 Oct. was treated for 0, 3, 6, 9, and 12 weeks of CS. The number of days to final germination and germination percentage were presented. Another group of *C. coreana* seeds harvested on 18 Oct. was treated for 0, 3, 6, 9, and 12 weeks of CS and the germination percentage and number of seeds that germinated was recorded.

After CS, pots were placed in the growth chamber maintained at 21/18 °C and the number of seeds germinated was recorded at 1 to 3 day intervals until no additional seeds germinated consecutively for 14 d. Treatment was replicated twice with 100 seeds per replication. Data were subjected to ANOVA with harvest dates and the duration of CS as variables, and the means were compared by Tukey's honestly significant difference test at 1% significance level.

Results and Discussion

Germination of *C. gotoana* seeds influenced by seed harvest dates (maturity) and X-ray images (Expt. 1). Fruits of *C. gotoana* harvested on 12 July were green and very tight and seedcoats in most of the seeds were milky yellow. Only one seed weighing 14.3 mg sank. The weight of seeds

harvested on or after 6 Sept. that sank was greater than 18.9 mg per seed, heavier than those that floated, which ranged between 14.3 and 16.3 mg (Table 1). The seed weight increased with delay in harvest dates. The percentage of seeds that sank increased significantly when seeds were harvested on 6 Sept. (59%) or 10 Oct. (73%).

Corylopsis gotoana seeds that were harvested on 1 Oct. and did not receive any CS germinated up to 25% for those that sank and 20% for those that floated (Table 1). The difference in the germination of seeds that sank and floated in the first week did not differ significantly, especially when seeds were harvested between 6 Sept. and 1 Oct. The final germination percentages following 3 months of CS were unaffected by whether seeds sank and floated, perhaps due to a low percentage of seeds that sank when seeds were harvested on 12 July. Late harvested seeds that floated germinated at 41% (10 Oct. harvest) to 54% (20 Sept. harvest); however, percentages were significantly lower than the percentages of seeds that sank.

Morphological differences related to the development of fruits were not recorded. However, based on the seed weight, the number of seeds that sank, and germination percentages, the 3 month harvest period clearly covered development stages ranging from immature seeds before 22 Aug. to mature seeds with fully developed internal structures (Kim et al., 2017) harvested on or after 6 Sept. If seeds are harvested and germinated as early as possible and grown under favorable environmental conditions, larger seedlings could be forced to flower in the next season one year after transplanting seedlings.

Immersing *C. gotoana* seeds for 20 min may not effectively separate fully developed seeds that sink from empty seeds nor accurately estimate the germination percentages. Increasing the immersion time up to 16 h increased the percentage of seeds that sank and improved the seed germination in both *C. coreana* and *C. sinensis* var. *calvescens* (Kim et al., 2017). *Corylopsis* seeds are very small and using the floating technique for 16 h or longer facilitates separation of full and

Table 1. Percentage of seeds that sank when immersed in water, weight of each seed, and germination percentages according to different harvest dates in *Corylopsis gotoana*.

Harvest date	Seeds that sank (%)	Wt of seed (mg)		Germination (%) ²			
		Sank ^y	Floated	Week 1		Final	
12 July	0 d ^a	14.3 d	13.2 c	0 d	3 d	0 c	50 a
2 Aug.	9 d	15.1 c	13.5 c	1 d	6 c	24 b	44 b
22 Aug.	46 c	17.0 b	13.6 c	12 b	6 c	73 a	52 a
6 Sept.	59 b	18.9 a	14.3 b	7 c	7 c	82 a	41 b
20 Sept.	61 b	19.0 a	14.7 b	14 b	14 ab	68 a	54 a
1 Oct.	69 ab	19.2 a	15.3 a	25 a	20 a	69 a	50 a
10 Oct.	73 a	19.5 a	16.3 a	23 a	12 b	75 a	41 b
Level of significance							
Harvest time – quadratic effect	***	***		***		***	
Sank vs. Floated seeds	—	***		NS		NS	

²Germination data were collected 1 week after sowing (week 1) without CS and following 3 months of CS (final on 19 June).

^ySeeds were immersed in water for 20 min and those that sank and floated were collected separately.

³Mean separation within columns by Duncan's multiple test. Means with different letters in a column are not significantly different from each other

NS, ***Non-significant or significant at $P \leq 0.001$, F test.

mature seeds from immature and empty seeds on a large scale, especially when X-ray scanning is not available. Immersing *Casuarina equisetifolia* seeds in petroleum (Sivakumar et al., 2007) or Crimean pine [*Pinus nigra* Arn. ssp. *pallasiana* (Lamb.) Holmboe (Avsar, 2010) in ethanol suggest that separation of *Corylopsis* seeds that can germinate should further be evaluated by testing various organic solvents.

The weight of seeds that sank cannot be used to determine when to harvest mature seeds, perhaps due to great variability in weight among individual seeds. A few seeds appeared to be semifull as revealed by X-ray images (Kim et al., 2017). X-ray images of seeds harvested on 6 Sept. (Fig. 1) are not very clear compared with images of castor bean (*Ricinus communis* L.) seeds (Carvalho et al., 2010). The

possible reasons include enlargement of small *Corylopsis* seeds or resolution of the device compared with the castor bean study. Differences in the imaging conditions may also play a role following exposure to 20kV for 15 s in this study compared with 20–50kV for 15–75 s for castor bean. Seeds that were categorized as semifull may either sink or float depending on the degree of development of internal structure. Seeds that were harvested early on 12 July (Fig. 2, Frame A) showed as emptier than full or semifull seeds which can be used to determine the seed quality as reported for castor beans (Carvalho et al., 2010). Delay in harvest date to 10 Oct. (Frame G) resulted in only four empty seeds and one semifull seed while the remaining 69% of the seeds were full seeds. The relationship between the weights of seeds,

the percentage of germinating seeds, and seeds showing fully developed internal structures warrants careful scrutiny, since a few floated seeds germinated, and also due to difficulties in classifying empty, semifull and full seeds based on X-ray images of seeds harvested in July and August.

Germination of C. coreana seeds influenced by seed harvest dates (maturity) and duration of cold treatment (Expt. 2). *Corylopsis coreana* seeds that sank following immersion into water were used for germination tests to evaluate the effect of seed harvest date (maturity) and CS for up to 12 weeks at 3-week intervals. Regardless of seed harvest date, seeds failed to germinate without CS and less than 15% of seeds germinated when given 3 weeks of CS (Fig. 3). When seeds were harvested on or after 15 Sept., germination was promoted by 9 weeks of CS to higher than 55%. Regardless of seed harvest dates, more than 60% of the seeds germinated when given 12 weeks of CS.

Increasing the duration of CS also accelerated the germination (Fig. 4) from 25 and 26 d following 3 to 6 weeks of CS, to less than 19 d when given CS for 9 or 12 weeks. Without CS, seeds achieved only 8% germination in 44 d, and germination started from 26 to 27 d after sowing (Roh et al., 2008). Upon 12 weeks of CS, the germination plateau of 69% was reached within ~29 d.

Conclusions

Based on the percentage of seeds that sink and germinate, *C. gotoana* seeds can be

Fig. 1. X-ray images of *C. gotoana* seeds harvested on 6 Sept.; empty seed showing only seedcoat (arrow), semifull seeds with deformed or partially full and blemished internal structure, and full and opaque seed with developed internal structure. Approximate size of seeds: Bar = 1 mm.

Fig. 2. X-ray images of *C. gotoana* seeds harvested on 12 July (frame A), 2 Aug. (frame B), 22 Aug. (frame C), 6 Sept. (frame D), 20 Sept. (frame E), 1 Oct. (frame F), and 10 Oct. (frame G). In Frame B, empty seeds (solid arrow) that floated when harvested early rather than late with more full seeds that sank (dotted arrow). Semifull seeds are indicated by double-headed dotted arrow. More empty and semifull seeds were observed when seeds were harvested early (Frames A>B>C) and full seeds when harvested late (Frames D<E<F<G).

Fig. 3. Number of days to final germination and final germination percentage of *C. coreana* seeds harvested at different dates as influenced by the number of weeks at 5 °C. Bar indicates significant different level by honestly significant difference at 5% level, F test.

Fig. 4. Germination of *C. coreana* seeds harvested on 18 Oct. as influenced by the number of weeks at 5 °C.

harvested as early as 22 Aug. and *C. coreana* around 15 Sept. to obtain nearly 70% germination. Therefore, it is concluded that mature seeds of *C. coreana* and *C. gotoana* can be harvested on or around 6–20 Sept., about 20–22 weeks after anthesis in mid-March. In *Styrax japonicus*, mature seeds that subsequently germinated were harvested 12 to 16 weeks

after anthesis (Roh et al., 2004). To maximize germination, longer than 6 weeks of CS is desirable, although a few *C. coreana* and *C. gotoana* seeds germinate without CS treatments.

Immersing *C. gotoana* seeds in water for 20 min did not effectively separate fully developed seeds from empty seeds, since more than 20% of seeds harvested on 1 Oct. that floated had germinated. Seed weight cannot be used to determine maturity. Mature *C. gotoana* seeds that sank upon immersing in water showed translucent X-ray images, representing well-developed internal structure when seeds were harvested on or around 6 Sept. Without any cold treatment, seeds germinated regardless of seed maturity showing >14% or >23% when seeds were harvested on or after 20 Sept. Longer than 6 weeks of CS was required to accelerate the germination and germination percentages of *C. coreana* seeds harvested on or after 5 Sept. Germination percentage of mature seeds harvested on 18 Oct. was increased to >72% as the duration of CS was increased to 12 weeks. Mature *C. coreana* seeds can be

harvested on or after 5 Sept. Six weeks of CS accelerates the germination and increases the germination percentage. Most interestingly, partial germination of seeds that were not treated with CS suggests that *Corylopsis* seed dormancy is not deep. Further investigations are needed to develop efficient methods to separate fully developed and mature seeds from partially developed or empty immature seeds.

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