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Germination of *Corylopsis* seeds evaluated by X-ray imaging and cold stratification

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Abstract

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Effects of immersing seeds in water and X-ray imagining on germination of *Corylopsis* seed were investigated. Seeds that sank or floated were collected after immersing in water for 5 min, 4 h or 16 h, and germinated with or without 5°C cold stratification (CS). In *C. coreana* and *C. sinensis* var. *calvescens*, 52% and 89%, respectively, of seeds that sank immediately developed embryos and cotyledons germinated. Germination of *C. sinensis* var. *calvescens* seeds that sank after 16 h increased to 45% after the first CS treatment, as compared to 12% without CS. Immersing seeds in water for 16 h to collect full and mostly full seeds is recommended. X-ray imaging coupled with immersion is effective technique to select viable seeds. Dormancy could be partially released by immersing seeds in water for 16 h; however, germination was improved after CS.

Keywords: *Corylopsis coreana*; *Corylopsis sinensis* var. *calvescens*; full seeds; seed dormancy; viable seed

The genus *Corylopsis* Siebold & Zucc. belongs to the Hamamelidaceae, and most taxa within the genus are native to China, Japan, and Korea. *Corylopsis coreana* Uyeki, *C. sinensis* var. *calvescens* Rehder & E.H. Wilson, and *C. gotoana* Makino were used quite commonly as landscape plants for their showy yellow flowers and early flowering characteristics. *Corylopsis* was propagated *in vitro*; however, its acclimatization was difficult (Suh, unpublished 2002), and, therefore, seeds were used for mass propagation.

It is desirable to test only viable seed lots to maximize germination percentage. In *Pinus nigra* ssp. p*allasiana,* full and viable seeds were separated from empty and non-viable seeds by immersing the seeds in 96% ethanol solution, and the full seeds that sank germinated (Avsar 2010). Germination of *Casuarina equisetifolia* L. seeds that sank in petroleum was increased; most seeds that sank were full rather than shrivelled, empty or insect damaged, as revealed by X-ray imaging analysis, yielding a low germination in Castor bean (*Ricinus communis* L.) seeds (Carbalho et al. 2010). *Corylopsis coreana* and *C. sinensis* var. *calvescens* seeds exhibited partial germination at 10°C without cold stratification (CS), but 2 month of CS was required for more than 90% of seeds to germinate (Kim et al. 2015; Roh et al. 2008). To break seed dormancy in many woody genera, stratification of seeds at around 1–5°C (CS) (Dirr 1990) was effective. In *Corylopsis*, it was not determined whether dormancy was imposed by the seed coat, or by internal conditions of the fully

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mature embryo (Baskin, Baskin 2004; Kim et al. 2015).

Objectives of these experiments were to study the seed germination of *C. coreana* and *C. sinensis* var. *calvescens* by investigating the feasibility of immersion of seeds in water for various durations to separate full and viable seeds from empty seeds and (b) to estimate the seed germination percentage based on of the development of embryo and cotyledon, as determined by X-ray images. The effect of cold stratification (CS) on the rate and speed of germination was also investigated.

MATERIAL AND METHODS

Plant materials and seed germination treatments. Seeds of *C. coreana* and *C. sinensis* var. *calvescens* harvested from field-grown plants at Beltsville, USA were evaluated. Seeds were immersed in water to separate seeds that sank from those that floated and were sown separately in 12.5 cm pots filled with Metro Mix 200 (SunGro Horticulture, Bellevue, USA) and received temperature treatments as specified in each experiment. Seeds were germinated in an air-conditioned greenhouse maintained at 20°/18°C (day/night).

Seed germination as affected by immersing seeds in water, X-ray imaging, and cold stratification (CS) treatments. Seeds of *C. coreana* and *C. sinensis* var. *calvescens* harvested on Oct. 21, 2011 were immersed in water and gently stirred using a glass rod. Seeds that sank in 5 min (sink imm.), 4 h (sink 4 h) or 16 h (sink 16 h) were collected separately, along with those that did not sink within 16 h (float 16 h).

Before the germination, seeds were glued onto index cards using Elmer's glue and imaged at the Ornamental Plant Germplasm Center at the Ohio State University, USA using a Faxitron MX-20 (Faxitron Co., Wheeling, USA). X-ray digital images were collected following exposure to 20kV for 15 seconds. After imaging, seeds were sown individually into 144-cell plug trays (Dillen Products, Middlefield, USA) filled with Pro-Mix BX on Nov. 17 (day 270). Trays were stored at 21°/18°C in a dark growth chamber and watered as needed.

Based on the X-ray images, seeds were classified into 4 categories (Fig. 1): fully developed embryo and cotyledons (full seeds); developed embryo and cotyledons showing a cavity (mostly full seeds); evident cavity between seed coat and shrivelled cotyledons and poorly developed embryo (partially empty seeds); and empty seeds showing shrivelled cotyledons without an embryo (empty seeds).

The number of seeds germinated was recorded at 3- to 4-day intervals. After 30 consecutive days without additional germination, trays were moved on Jan. 17, 2012 to a dark cooler (day 89) for the first 30 days of CS at 5°C (1 CS), and were then moved to the greenhouse maintained at 21°/18°C on Feb. 19 (day 122). After 30 days without additional germination, trays were moved back for a second CS treatment (2 CS) on Mar. 18 (day 149), and transferred back to the greenhouse for the final time on May 3 (day 195). Germination was record-

Fig. 1. X-ray images of *Corylopsis coreana* seeds: Fully developed embryo and cotyledons (full seeds (a), developed embryo and cotyledons showing a cavity (mostly full seeds) (b), evident cavity between seed coat and shrivelled cotyledons and poorly developed embryo (partially empty seeds) (c), and empty seeds showing shrivelled cotyledons without an embryo (empty seeds) (d); bar = 4.5 mm

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ed for another 45 days (day 239). Treatments were replicated twice with 50 seeds per replication.

Experimental design and data analysis. Data were subjected to analysis of variance using the Statistical Analysis System software ver. 9.0 (Statistical Analysis System, 2002). *Corylopsis* taxon, CS treatment and seed development classification were included as variables. Significant differences were observed by taxon, so data were analysed again separately by taxon means comparison that was conducted using the Tukey's honest significant difference (HSD) test or Duncan's Multiple Range test as indicated in each table.

RESULTS

Seed germination as affected by immersing seeds in water, X-ray images, and cold stratification (CS)

In *C. coreana* and *C. sinensis* var. *calvescens*, 85% and 91%, respectively, of seeds that sank immediately were classified as full (Fig. 1), while 14% and 7% of seeds were partially full (Table 1). More than 83% of seeds in both species that floated after immersing seeds in water for 16 h were empty. Without CS, 11% of *C. coreana* and 12% of *C. sinensis* var. *calvescens* seeds that sank immediately germinated in 33 days and 49 days, respectively (Table 2). Germination was significantly increased to 19% and 23% for *C. coreana* after the first (1 CS) and second CS (2 CS) treatment, respectively; comparable values for *C. sinensis* var. *calvescens* were 45% and 30%.

Germination percentages of *C. coreana* seeds that sank in 16 h and *C. sinensis* var. *calvescens* seeds that sank in 4 h did not differ significantly from those seeds that sank immediately. After 1 CS, germination percentage of *C. sinensis* var. *calvescens* seeds was significantly increased to 44–45% (sink imm. and 4 h sink) as compared to 12% for seeds with 0 CS. After 2 CS, germination was significantly lower in comparison with 0 CS for *C. sinensis* var. *calvescens* seeds that sank in 4 or 16 hours. Germination rates were lower for seeds that floated than for those that sank. Five percent or less of seeds that floated after 16 h of immersion in water germinated in both species.

Number of days to germination was accelerated by CS in both taxa. However, in comparison with germination rates of seeds that sank immediately, no differences were observed for seeds immersed in water for 16 h in *C. coreana* and for 4 h in *C. sinensis* var. *calvescens* (Table 2). However, when seeds were immersed in water for 16 h, germination was significantly accelerated when seeds received 0 CS or 1 CS as compared to seeds that sank immediately; from 33 days to 26 days in *C. coreana* and from 49 days to 37 days in *C. sinensis* var. *calvescens.*

Table 1. Percentage of seeds of *Corylopsis coreana* and *C. sinensis* var. *calvescens* classified into each of four development categories based on X-ray images following immersing seeds in water for various durations

Immersing in water[1]	Image classification							
	C. coreana				*C. sinensis* var. *calvescens*			
	full	mostly full	partially empty	empty	full	mostly full	partially empty	empty
Sink imm.	85	14	1	0	91	7	1	1
4 h sink	–	–	–	–	71	15	10	4
16 h sink	74	16	6	4	89	4	7	0
16 h float	7	4	6	83	0	7	7	86
Level of significance[2]								
Immersing duration (ID)		**				*		
Image classification (IC)		**				**		
ID × IC		*				*		
HSD at $P \leq 0.01$		8.3				6.4		

[1]seeds were immersed in water for 5 min, 4 h, or 16 h, and seeds that sank immediately (Sink imm.) or after 4 h or 16 h were collected separately; [2] **, * – significant at $P \leq 0.01$ and $P \leq 0.05$, *F*-test

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Table 2. Germination percentage and speed of germination of seeds of *Corylopsis coreana* and *C. sinensis* var. *calvescens* as affected by immersing in water and cold stratification

Cold stratification (CS)[1]	Taxa						
	C. coreana			*C. sinensis* var. *calvescens*			
	sink imm.[2]	16 h sink	16 h float	sink imm.	4 h sink	16 h sink	16 h float
	Germination percentage						
0 CS	11	19	1	12	12	44	5
1 CS	19	16	2	45	44	6	0
2 CS	23	15	2	30	2	0	0
Germination of full and mostly full seeds[3]	52	47	29	89	67	53	7
Level of significance[4]							
CS	**			**			
Seed classification	**			**			
CS × Seed classification	ns			*			
HSD $P \leq 0.01$	5.4			7.6			
	Number of days to germination[e]						
0 CS	33	39	26	49	46	37	46
1 CS	118 (28)[5]	112 (28)	111 (27)	111 (27)	107 (23)	103 (19)	–
2 CS	184 (25)	184 (25)	184 (25)	184 (25)	181 (22)	–	–
Level of significance							
CS	*			*			
Seed classification	*			*			
CS × Seed classification	ns			ns			
HSD $P \leq 0.05$	5.1			6.9			

[1]after sowing, seeds were germinated, then moved to 5°C for the first 30 days of CS treatment (1 CS) treatment, and then moved to a greenhouse to continue seeds germination, and then exposed to a second CS treatment (2 CS); [2]seeds were immersed in water for 5 min, 4 h or 16 h, and seeds that sank immediately (sink imm.) or after 4 h or 16 h were collected separately; [3]full seeds with developed embryo and cotyledons and mostly full seeds with developed embryo and cotyledons showing a cavity (X-ray images – Fig 1); [4] ns,**, * – non-significant, significant at $P \leq 0.01$ and $P \leq 0.05$, *F*-test; [5]number of days to germination were counted from Nov. 17, 2011, when seeds were sown, or after the completion of 1 CS or 2 CS (in parenthesis)

Relationship between embryo and endosperm development analysed by X-ray images and seed germination

Seeds were assigned to one of 4 development categories based on X-ray images: full seeds, mostly full seeds with a cavity, partially empty seeds, and empty seeds (Fig. 1). When each full and mostly full seed of *C. coreana* was matched with the germination data, 52% of seeds that sank immediately (Fig. 2, upper image) germinated, as compared to 47% germination for seeds that sank after 16 h of immersion (Table 2). Without CS, most full seeds (Fig. 2, indicated with A) had germinated within 184 days. Some mostly full seeds (indicated B) germinated within 184 days after receiving 2 CS. Mostly full seeds with a cavity as indicated with B (Fig. 2, upper frame) germinated in 43 days without CS or 183 days after 2 CS. Overall, partially empty seeds (indicated with C) did not germinate. No empty seeds (indicated with D) germinated, regardless of density.

Three full seeds of *C. coreana* that floated after 16 h in water did not germinate, although one seed germinated after each of the following treatments: 0 CS (29 days to germinate), 1 CS (99 days to germinate), and 2 CS (184 days to germinate). A similar trend was also observed in *C. sinensis* var. *calvescens* (data not presented).

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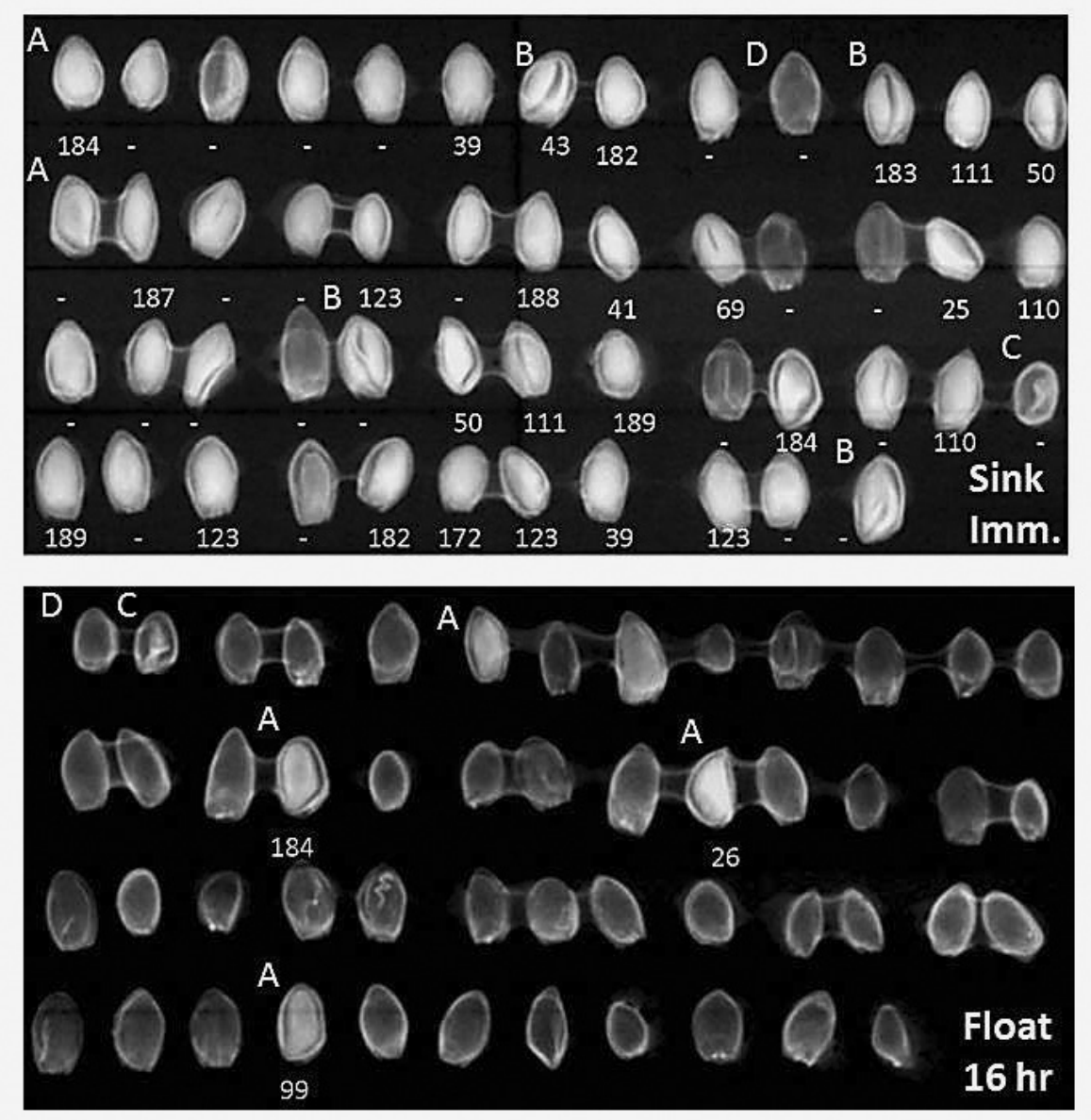

Fig. 2. X-ray images and number of days to germination of *Corylopsis coreana* seeds that sank immediately (upper image) or floated after immersion in water for 16 h (lower image). Number below the image of an individual seed indicates the number of days to germinate. Seeds marked as A, B, C, and D were full seeds, mostly full, partially empty, and empty, respectively. Seeds marked as A or B showed great discrepancies in germination, i.e., did not germinate, or germinated with or without stratification at 5°C. Seeds marked as C or D did not germinate

DISCUSSION

The duration of immersing seeds in water and seed germination

Full seeds of *C. coreana* and *C. sinensis* var. *calvescens* that are able to germinate are effectively separated from empty seeds following immersion in water and using X-ray imaging. Germination percentage of *C. sinensis* var. *calvescens* seeds that sank in 16 h is improved, and is further increased by CS treatment for seeds that sank in 4 hours. It is suggested that some metabolites related to seed germination, e.g., inhibitor(s), may be leached out from *C. sinensis* var. *calvescens* seeds by immersing seeds for 16 hours. Number of days to germination was accelerated by CS in both taxa. Therefore, it is concluded that immersing *Corylopsis* seeds effectively separates seeds and improves germinate percentages, which are further improved by CS treatment.

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Relationship between categories evaluated by X-ray images and seed germination

Among the many techniques used to separate viable seeds that germinate from non-viable seeds, X-ray imaging is considered as a cost-effective and convenient technique (Avsar 2010). Lack of clear and distinct images for some *Corylopsis* seeds may have been due to the small size of seeds, and perhaps from radiation conditions (20 Kvp and 20 s exposure).

Based on the X-ray images, full *Corylopsis* seeds with well-developed embryos and cotyledons and some mostly full seeds showing cavities are considered viable and will germinate, whereas partially empty and empty seeds do not germinate. However, a few full and mostly full seeds of both *Corylopsis* taxa failed to germinate. In a previous study with *Cunninghamia* and *Cryptomeria*, all full seeds germinated, while empty seeds did not (Li et al. 1999).

A few mostly full *Corylopsis* seeds with obvious cavities germinated without showing any abnormal growth of seedlings (data not presented). The increase in the cavity area observed in partially empty seeds may suggest that embryos and endosperm are not fully developed or are degenerated, as reported in European larch (Kosiński 1987). Immersing seeds in water coupled with X-ray imaging is a feasible and potentially cost-effective means to select small, viable seeds on a large scale, as compared to magnetic resonance imaging as investigated with *Styrax japonicus* L. (Roh et al. 2004).

The nature of *Corylopsis* seed dormancy

The nature of dormancy in *Corylopsis* seeds has not been well understood. *Corylopsis* seeds germinated without any CS in this study, although *C. glabrescens* seeds required 3 to 5 months of warm stratification (WS) at 21°C followed by 3 months of CS (5°C) (Dirr 1990). The ability of seeds of *Corylopsis* to germinate without any CS in this study agrees with the previous report by Roh et al. (2008). Seeds of *Corylopsis coreana*, *C. sinensis* var. *calvescens* and *C. gotoana* do not need 3- to 5-months of WS. Rather, these findings suggest that they need less than 3 months of CS.

Since physical dormancy imposed by the seed coat is one of the possible reasons for dormancy as stated by Baskin and Baskin (2004), it is likely that immersion of seeds in water releases seeds of *Corylopsis* from dormancy. Immersing seeds of *C. sinensis* var. *calvescens* in water for 16 h increases the number of full seeds that germinate without CS, suggesting that dormancy inducing substance(s) present in the seed coat may have been leached out. This requires further investigation by testing the leachates on seeds that do not normally exhibit dormancy. It is recommended that *Corylopsis* seeds be immersed in water longer than 16 h to see whether germination percentages can further be increased.

CONCLUSION

Percentage of seeds of *C. sinensis* var. *calvescens* that sank in water for 16 h germination were increased to 45% after the first CS (1 CS), as compared to 12% without CS. Based on the X-ray images and immersing seeds in water, most of the full and mostly full seeds of *C. coreana* and *C. sinensis* var. *calvescens* germinated. Furthermore, X-ray imaging may be very useful to separate viable seeds from non-viable seeds on a large scale. Based on seed weights, X-ray images, and germination percentages for seeds harvested over time, it is concluded that seeds harvested after Sept. 20 are mature. Dormancy in *Corylopsis* is composed of physiological dormancy imposed by the embryo/endosperm, which can be overcome without CS, and physical dormancy imposed by the seed coat, which can be released by immersing seeds in water for 16 hours. These two types of dormancy may be involved in germination, especially in *C. sinensis* var. *calvescens*. A few seeds germinate without CS; however, to increase germination, 2 months of CS at 5°C treatment is recommended.

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