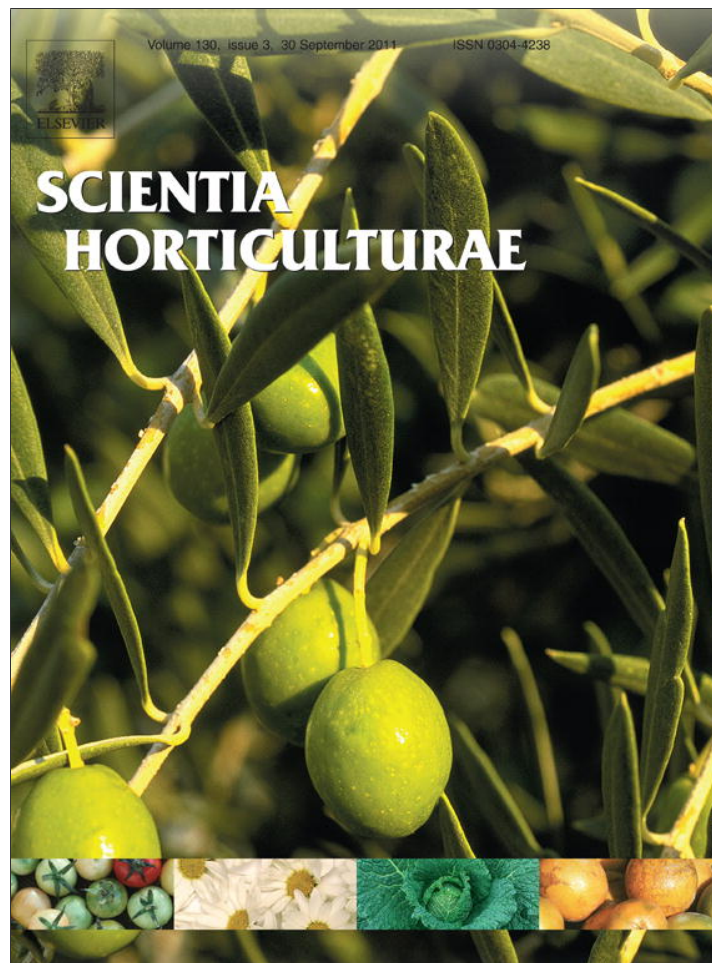


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Optimized scarification protocols improve germination of diverse *Rubus* germplasm

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ARTICLE INFO

Article history:

Received 23 June 2011

Received in revised form 3 August 2011

Accepted 11 August 2011

Keywords:

Dormancy

Germplasm

Hilar-end hole

Seed coat

Seed treatment

Tetrazolium testing

ABSTRACT

Seed collections of the wild relatives of cultivated blackberry and raspberry (*Rubus* species) are maintained at the National Clonal Germplasm Repository, Corvallis, OR. Information on wild species germination requirements is rarely available, and germination may be poor or slow, making it difficult for scientists to use them for breeding improved cultivars. Eight diverse *Rubus* species in 6 of the 12 *Rubus* subgenera from seed stored at -20°C for 1–23 years were studied. Seed weight, seed-coat thickness and hardness varied widely. Scarification with sulfuric acid (98% H_2SO_4) or sodium hypochlorite (14% NaOCl) was followed by germination treatments of deionized water (DI), smoke gas or a combination of gibberellic acid (2.03 mg/L GA_3) and potassium nitrate (34 mg/L KNO_3) during stratification. The commonly used scarification protocols were not effective for many species; but effective scarification exposure was established based on the amount of embryo damage seen with 2,3,5 triphenyl tetrazolium chloride (TZ) viability testing. H_2SO_4 scarification followed by a treatment with KNO_3 and GA_3 during stratification was highly effective for the most species. Two species in subgenus *Anoplobatus* had a hilar-end hole that allowed rapid germination of unscarified seed. Some species with extremely hard seed coats had little or no germination, and longer scarification times are suggested based on seed size, seed-coat thickness and hardness and viability testing.

Published by Elsevier B.V.

1. Introduction

Germplasm collections are valuable to plant breeders as a source of new genes or traits to improve cultivated crops. The National Clonal Germplasm Repository (NCGR) in Corvallis, OR preserves more than 1300 wild-collected *Rubus* seed accessions at -20°C . These seeds are available to plant breeders for blackberry and raspberry crop improvement, however little is known about the germination requirements of most of the species. Often they do not germinate using the commonly used protocols found in the literature. In addition the seed collections were made by many plant collectors over many years and information on the seed maturity and initial viability is often not known. The genus *Rubus* is very diverse, includes over 750 species in 12 subgenera, and is found on all continents except Antarctica (Finn, 2008). The deep dormancy of many *Rubus* species seeds makes it difficult to use wild germplasm for blackberry and raspberry breeding programs (Clark et al., 2007; Daubney, 1996). Seed germination of the resulting hybrids is one

of the largest challenges in blackberry breeding as there is large variation in germination based on genotype (Clark et al., 2007).

Rubus seed germination is constrained by both physical and physiological dormancy. An impermeable seed coat imposes mechanical resistance to growth and may contain chemical inhibitors; slow germination may also be due to the slow maturation of the dormant embryo (Zasada and Tappeiner, 2003). The endosperm and testa of dormant blackberry seeds contain growth inhibitors that degrade or are leached out during stratification under moist conditions, resulting in breaking dormancy and germination after about 5 months (Lasheen and Blackhurst, 1956). Polyphenols in the seed coat may also be linked with seed dormancy and longevity (Werker et al., 1979). Heit (1967b) noted that a hard seed coat or the combination of a hard seed coat and a dormant embryo will inhibit germination.

Sulfuric acid (98% H_2SO_4) scarification and a long stratification period, first warm and then cold, are required for satisfactory germination of hard-seeded species (Heit, 1967a). A scarification procedure using concentrated H_2SO_4 neutralized with calcium hypochlorite ($\text{Ca}(\text{ClO})_2$) and calcium hydroxide ($\text{Ca}(\text{OH})_2$) described by Jennings and Tulloch (1965) is a widely used protocol for *Rubus* seed germination. H_2SO_4 scarification of 30 min is recommended for the small seeded raspberries (*Rubus idaeus* L., *Rubus occidentalis* L.) and up to 3 h for the larger seeded

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Table 1Taxonomy, year collected, origin and identifying numbers of cold-stored seed of 8 *Rubus* species from the USDA-ARS National Clonal Germplasm Repository collections.

<i>Rubus</i> species	Subgenus	Year collected	Origin	Local number	PI number
<i>R. arcticus</i> subsp. <i>stellata</i>	<i>Cyclactis</i>	1996	U.S.A.	1881	606514
<i>R. chamaemorus</i> L.	<i>Chamaemorus</i>	2001	Russian Federation	2156	638202
<i>R. leucodermis</i> Douglas ex Torrey & A. Gray	<i>Idaeobatus</i>	1985	U.S.A.	651	553681
<i>R. niveus</i> Thunb.	<i>Idaeobatus</i>	2007	Brazil	2354	653286
<i>R. odoratus</i> L.	<i>Anoplobatus</i>	2003	U.S.A.	2214	638246
<i>R. parviflorus</i> Nutt.	<i>Anoplobatus</i>	2007	U.S.A.	719	553805
<i>R. sanctus</i> Schreber	<i>Rubus</i>	1989	Pakistan	1053	553879
<i>R. urticifolius</i> Poir.	<i>Lampobatus</i>	1990	Ecuador	1290	548933

blackberries (*Rubus* spp.) (Hummer and Peacock, 1994). Scarification with sodium hypochlorite (15% NaOCl) is recommended for sand blackberry seeds (*Rubus cuneifolius* Pursh) (Campbell et al., 1988). Due to the great diversity of *Rubus* species, a single standard protocol is unlikely to be useful for germinating seed of wild germplasm. This genus is especially difficult due to the diversity in seed-coat thickness and structure present in the genus (Daubney, 1996). In standard protocols *Rubus* seed are usually stratified and germinated in sand, sphagnum or soil in a greenhouse (Clark et al., 2007; Hummer and Peacock, 1994), but can also be germinated *in vitro* (Mian et al., 1995) or on blotters (Wada, 2009). After effective scarification is completed, additional treatments can also impact the rate and amount of germination. Some *Rubus* species germinate well with only water added to the medium, while others have increased germination when smoke from cellulose fires or gibberellic acid and potassium nitrate are added during stratification and germination (Wada, 2009; Wada and Reed, submitted for publication).

The objective of this study was to determine the germination response of wild-collected cold-stored *Rubus* germplasm held in the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), National Clonal Germplasm Repository (NCGR), Corvallis, OR. Seed attributes of eight species were delineated and commonly used H₂SO₄ and NaOCl scarification treatments were tested for reducing physical dormancy. We also tested the effects of germination treatments for overcoming physiological dormancy and improving germination.

2. Materials and methods

2.1. Plant materials

The NCGR *Rubus* seed-collection database was screened and 8 species belonging to six subgenera were selected from the germplasm collections stored for 1–23 years at –20 °C (Table 1). Accessions were selected with seed of variable sizes that had 30–95% TZ viability and a large amount of seed, so that seeds of the accession were still available for distribution. *Rubus leucodermis*, *Rubus niveus*, *Rubus odoratus*, and *Rubus parviflorus* were used for four germination treatments (750 seed each) and four species with fewer available seed (*Rubus arcticus*, *R. chamaemorus*, *Rubus sanctus*, and *Rubus urticifolius*) were used for two germination treatments (450 seed each).

2.2. Seed-coat thickness and hardness

Seed-coat thickness was measured for ten seed of each species. A Nikon SMZ 1000 stereomicroscopic Zoom Microscope (Nikon Instruments, Tokyo, Japan) was employed and measured with Infinity image capture and analysis software (Luminera Corporation, Ottawa, Canada). Measurements were taken in the center of the cut seed equidistant from the micropylar region and the hilar end. Hardness ratings of 1–5 were assigned after seed samples were soaked in deionized (DI) water for 2 days and hand sectioned with

a scalpel. The subjective hardness grading was 1 soft, 2 slightly hard, 3 hard, 4 very hard (difficult to cut), and 5 extremely hard (very difficult to cut). This scale was developed based on sampling of a range of species.

2.3. Microscopy

Light microscope (LM) images were taken using a Nikon SMZ 1000 stereomicroscopic Zoom Microscope (Nikon Instruments, Tokyo, Japan). Scanning electron microscopy (SEM) images were taken using an AmRay3300 (Amray, Bedford, MA)

2.4. Scarification procedures

Due to the lack of specific guidelines for diverse species, scarification times were estimated for each accession based on the subgenus and seed weight. (1) Control—no treatment. (2) concentrated sulfuric acid (98+% H₂SO₄) in an ice bath for 30 min–3 h, then rinsed in running water for 1 h. Then seeds were soaked for 5 min in a solution of 3 g/L Ca(ClO)₂ completely dissolved in water with 3 g/L Ca(OH)₂ and rinsed for 5 min in running water. Seeds were rubbed against a strainer before stratification to remove the carbonized portions of testa. (3) Sodium hypochlorite (14% NaOCl) at room temperature for 2–8 h and rinsed for 1 h in running water. Scarified seeds were placed on germination blotters for stratification and germination.

2.5. Secondary scarification test

After poor response to the initial germination tests with 1 h scarification, seeds of *R. chamaemorus*, one of the largest, thickest and hardest seeds, were H₂SO₄ scarified for 0, 3, 3.5, 4, 4.5, 5.0, and 5.5 h to determine if TZ testing could be used to optimize scarification duration. TZ tests were performed on the scarified seed after each time point to determine viability of the embryo and observe any damage resulting from the scarification process.

2.6. Germination treatments

Germination blotters were placed in 20 mm × 100 mm petri dishes and fully soaked with DI water. Scarified seed were placed on the blotters and 5 mL the germination treatment solution was added. Control seeds were not scarified and were germinated with only DI water. NaOCl scarified seeds were treated with only DI water. H₂SO₄ scarified seed treatments were: (1) DI water, (2) gibberellic acid (2.03 mg/L GA₃) plus potassium nitrate (34 mg/L KNO₃), or (3) smoke gas solution (Super Smoke Plus, Claremont, South Africa) prepared by soaking 5 pieces of smoke infused paper in 250 mL DI water for 24 h. All treatments were replicated with 50 seeds per 9 cm plastic Petri dish and 3 dishes per accession (*n* = 150).

Table 2
Seed characteristics, years stored and TZ viability of 8 cold-stored *Rubus* species from the USDA-ARS National Clonal Germplasm Repository collections.

<i>Rubus</i> species	Years stored	% TZ viability	Seed weight (g/100 seed) ^a	Seed-coat thickness (mm) ^b	Seed-coat hardness (1–5) ^c
<i>R. arcticus</i>	12	95	0.22 b	0.225 b	5
<i>R. chamaemorus</i>	7	80	0.86 a	0.238 a	5
<i>R. leucodermis</i>	23	75	0.11 cde	0.073 f	3
<i>R. niveus</i>	1	60	0.12 cd	0.088 e	3
<i>R. odoratus</i>	5	85	0.09 de	0.128 d	1
<i>R. parviflorus</i>	23	85	0.13 c	0.132 d	1
<i>R. sanctus</i>	19	30	0.21 b	0.133 d	3
<i>R. urticifolius</i>	18	60	0.09 e	0.149 c	3

^a Means in a column followed by the same letters are not significantly different calculated by Duncan's multiple range test ($\alpha = 0.05$) ($n = 100$, 3 replications).

^b $n = 10$.

^c Seed-coat hardness rating of 1 soft, 2 slightly hard, 3 hard, 4 very hard, and 5 extremely hard.

2.7. Stratification procedures

Seeds were held in a warm stratification ($18 \pm 1^\circ\text{C}$) in an incubator (Hoffman Manufacturing Inc., Jefferson, OR) for 1 month with a 16 h photoperiod ($25 \mu\text{M m}^{-2} \text{s}^{-1}$) followed by 3 month cold stratification (4°C) in the dark and finally in the germinator with 8 h dark at $15 \pm 1^\circ\text{C}$ and 16 h light at $30 \pm 1^\circ\text{C}$ for 3 months. All plates were placed in a randomized complete block design with each incubator shelf considered a block. Plates were sprayed with thiophanate methyl fungicide (3336 WP, Cleary Chemical Corporation, Dayton, NJ; 0.13 mL/L) as needed to control fungi.

2.8. Data set and analysis

Germination counts were taken monthly until there was no more germination (at 7 months). Raw data of germination counts were used for the statistic analysis, and percentages were presented in the figures and tables. Data were analyzed by ANOVA using SAS 9.2 (2008). Means were separated with Duncan's multiple range tests significant at $\alpha = 0.05$. Dunnett's test of pair-wise multiple comparisons significant at the 95% confidence limits level were also used for analysis of interactions among genotypes and treatments.

3. Results

3.1. Seed characteristics

TZ testing indicated high to moderate viability for all but one accession (Table 2). Weight of 100 seed ranged from as little as 0.09–0.86 g, a $9.5\times$ difference, and seed-coat thickness and hardness varied widely as well (Table 2). *R. chamaemorus*, the heaviest seed, had a very thick, extremely hard seedcoat, while *R. odoratus* and *R. urticifolius*, the two smallest seeds, had moderately thick, and soft to hard seed coats (Table 2). *R. odoratus* and *R. parviflorus* had soft seed coats and medium thickness. Seed-coat thickness varied from 0.073 mm (*R. leucodermis*) to 0.238 mm (*R. chamaemorus*).

3.2. Seed-coat anatomy

LM of *R. odoratus* and *R. parviflorus* seed coats revealed that each had a unique seed-coat structure, a hilar-end hole (Fig. 1) that was only apparent in these two species. Germination of these species was unusually early at 1 month (data not shown) and occurred at high rates. Unscarified seed of these species germinated as well as the scarified seed (Table 3).

3.3. Viability following scarification

The poor response of several thick coated seed led us to viability testing of the seed following increased scarification durations. TZ testing of *R. chamaemorus* seed indicated that control seed and seed scarified for up to 4 h were highly viable as shown by red stained embryos (Fig. 2A and B). H_2SO_4 scarification resulted in significant embryo damage at 4.5 h and severe embryo damage at 5 and 5.5 h when most of the seed was no longer viable (Fig. 2C).

3.4. Germination

No controls germinated for any of the 8 species at 1 month. At the end of the warm stratification (1 month) for scarified seed, only *R. urticifolius* had 53% germination with $\text{H}_2\text{SO}_4 + \text{GA}_3 + \text{KNO}_3$; *R. parviflorus* 34% with H_2SO_4 and smoke; and *R. odoratus* 12% with $\text{H}_2\text{SO}_4 + \text{GA}_3 + \text{KNO}_3$ (data not shown). At the end of the cold stratification (4 months) none of the controls had germinated, but scarified *R. sanctus* (28%) and *R. urticifolius* (74%) germinated with $\text{H}_2\text{SO}_4 + \text{GA}_3 + \text{KNO}_3$. There was little germination during cold stratification (2–4 months data not shown).

Seeds were held in the germinator for 7 months, but germination reached a maximum by 6 months. At 6 months the unscarified controls of *R. odoratus*, *R. parviflorus* and *R. sanctus* had moderate to good germination (50–72%) as did the scarified seeds (Table 3). *R. chamaemorus* had 2.7% germination of controls and the remaining

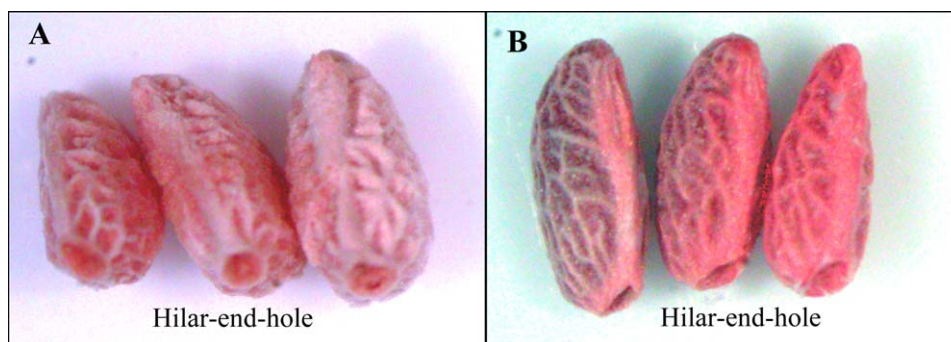


Fig. 1. Hilar-end hole on the seedcoat of two subgen. *Anoplobatus* species, *R. odoratus* L. (A) and *R. parviflorus* Nutt. (B). The micropyle is located on the opposite end of the seed.

Table 3Percent germination of cold-stored seed from 8 *Rubus* species 6 months after scarification and post-scarification germination treatments.

<i>Rubus</i> species ^a	Scarification ^b (h)		Unscarified control (%)	H ₂ SO ₄ (DI water)	H ₂ SO ₄ + GA ₃ + KNO ₃	H ₂ SO ₄ + smoke	NaOCl (DI water)
	H ₂ SO ₄	NaOCl					
<i>R. arcticus</i>	0.5	2	0.0 b	NT ^c	0.7 b	NT	2.7 a
<i>R. chamaemorus</i>	1	4	2.7 b	NT	14.0 a	NT	12.7 a
<i>R. leucodermis</i>	0.67	3	0.0 c	32.0 b	48.7 a	32.0 b	0.0 c
<i>R. niveus</i>	0.67	3	0.0 d	25.3 b	42.7 a	26.0 b	4.7 c
<i>R. odoratus</i>	0.5	2	72.0 ab	52.7 b	61.3 ab	52.0 b	86.7 a
<i>R. parviflorus</i>	0.67	3	42.7 a	39.3 a	56.7 a	68.7 a	40.7 a
<i>R. sanctus</i>	3	8	50.7 a	NT	36.7 a	NT	48.0 a
<i>R. urticifolius</i>	1	8	0.0 c	NT	96.7 a	NT	32.0 b

^a Means in a row followed by the same letters are not significantly different calculated by Duncan's multiple range test ($\alpha=0.05$) (3 replicates of 50 seeds, $n=150$).

^b Hours scarified based on initial assessment, not necessarily the optimal time; with H₂SO₄ on ice and NaOCl at room temperature.

^c Not tested due to limited amount of seed available.

species control seed did not germinate. *R. arcticus* and *R. chamaemorus* had trivial (<15%) or no germination on any treatment. *R. leucodermis*, *R. niveus*, *R. odoratus*, and *R. parviflorus* germinated on DI water after scarification with H₂SO₄ had $\geq 25\%$ germination. After NaOCl, scarification and DI water germination *R. odoratus*, *R. parviflorus*, *R. sanctus*, and *R. urticifolius* had <30% germination (Table 3).

All 8 species were tested with H₂SO₄ scarification and the GA₃ + KNO₃ germination treatment. Germination on this treatment was equal to or significantly better ($p < 0.05$) than the other treatments for the six species that germinated >36% (Table 3). The two species with low or no germination on all treatments (Table 3) were large seeds with thick and extremely hard seed coats (Table 2).

4. Discussion

The eight *Rubus* species from the NCGR collection are classified in six subgenera and were from samples collected from worldwide geographical regions and stored for 1–23 years at -20°C (Table 1). Seeds for these species arrived at NCGR through various routes, and the maturity of the seed, handling after collection and any prior storage information are often not well documented. The only study of *Rubus* seed longevity in cold storage was by Clark and Moore (1993) who studied open-pollinated blackberry (*Rubus* spp.) seeds after 22–26 years of cold storage at $4\text{--}5^{\circ}\text{C}$. They reported germination ranging from 0 to 84% with 3 h sulfuric acid scarification. As seeds age, both viability and germination inhibiting substances may diminish. Since scarification techniques are not well defined, this can also impact germination. It is difficult to draw conclusions on the effectiveness of germination tests for some of the stored species in our study because there were no initial germination tests and the conditions of collection and handling before cold storage at NCGR are often not known. As a whole, the best treatment for a wide range of species was scarification with H₂SO₄ and germination in the presence of GA₃ + KNO₃ (Table 3). This treatment produced >36% germination for 6 of 8 species and was as good as, or better than,

all other treatments (Table 3). Species with thin, soft seed coats appear to lose viability sooner than those with thicker, harder seed coats and this should be studied further to determine safe storage for these species.

Control seed produced moderate germination for *R. parviflorus* (43%) and *R. odoratus* (72%). Both are small seeds with moderately thick, soft seed coats and belong to subg. *Anoplobatus*. Differences between the types of scarification and among germination treatments were not significant for these species (*R. parviflorus*; $p=0.426$, *R. odoratus*; $p=0.1191$) (Table 3). Satomi and Naruhashi (1971) noted that the seed of *R. odoratus* had a unique hilum position compared to the other Japanese *Rubus* they studied and they emphasized that subg. *Anoplobatus* requires further investigation. Our earlier study of 57 *Rubus* species, utilizing light and scanning electron microscopy, revealed that both species have a unique hilar-end hole in the seed coat not found in the other subgenera (Wada and Reed, 2008). This unique structure is not the hilum, but is present at the tip of the seed on the end opposite the micropyle (Fig. 1) and provides an open channel for water imbibition, resulting in high germination of unscarified seed. None of the treatments produced a significant increase in the already moderate to high germination of the controls for these species (Table 3).

Recorded seed viability indicated by TZ (Table 2) was similar to the actual germination results of most genotypes (Table 3) and in the end indicated that more scarification was needed for some species. Assessing seed-coat thickness and grading hardness are important for proper scarification and the resulting germination response for the specific genotypes. Scarification in this study was based on subgenus and seed size, but not on the seed coat characteristics. The two species with low or no germination (Table 3) had large seeds and thick, extremely hard seed coats (Table 2). With the identification of seed-coat thickness and hardness as significant factors in germination response, it now appears obvious that these seeds required 2 to as much as 8 times longer scarification than we used in this study. Moore et al. (1974) found that blackberry hybrid seed averaged only 17% germination after a 3 h scarification



Fig. 2. *R. chamaemorus* embryos stained with tetrazolium (TZ) after prolonged H₂SO₄ scarification: (A) non-scarified (control), (B) 3 h scarified and (C) 5.5 h scarified embryos.

Table 4
Tested scarification and suggested times based on actual germination, seed size, seed-coat thickness and hardness of 8 *Rubus* species. Tested scarification times were based on subgenus and seed weight. Suggested times are based on weight, seed-coat thickness and hardness measurements.

<i>Rubus</i> species	Seed weight (g/100 seed) ^d	Seed-coat thickness (mm) ^c	Seed-coat hardness (1–5) ^b	H ₂ SO ₄ scarification (h)	
				Tested	Suggested
<i>R. arcticus</i>	0.22 b	0.225 b	5	0.5	3.5–4
<i>R. chamaemorus</i>	0.86 a	0.238 a	5	1	3.5–4 ^a
<i>R. leucodermis</i>	0.11 cde	0.073 f	3	0.66	1
<i>R. niveus</i>	0.12 cd	0.088 e	3	0.66	1
<i>R. odoratus</i>	0.09 de	0.128 d	1	0.5	0.5
<i>R. parviflorus</i>	0.13 c	0.132 d	1	0.66	0.5
<i>R. sanctus</i>	0.21 b	0.133 d	3	3	3.5–4
<i>R. urticifolius</i>	0.09 e	0.149 c	3	1	1

^a Actual time determined by TZ testing.

^b Seed-coat hardness rating of 1 soft, 2 slightly hard, 3 hard, 4 very hard, and 5 extremely hard.

^c $n = 10$.

^d Means in a column followed by the same letters are not significantly different calculated by Duncan's multiple range test ($\alpha = 0.05$) ($n = 100$, 3 replications).

of very-thick coated (0.47 mm) seeds. This combination of large seed, with a very-thick and very-hard seed coat indicates that *R. arcticus*, *R. chamaemorus* and the other similar seeds require more than a 3 h scarification to adequately reduce the seed coat and allow germination.

Peacock and Hummer (1996) germinated *R. chamaemorus* after a 0.5 h H₂SO₄ scarification (26%) and found no significant difference in germination compared to unscarified seed (32%). We increased the scarification to 1 h but observed only 14% germination, indicating insufficient scarification (Table 3). Scarification studies of *R. chamaemorus* seed quantified with TZ testing indicated that the 0.238 mm seed coat protected the embryo from damage from the H₂SO₄ scarification for 3–4 h (Fig. 2). Based on the available information, scarification treatments for other species were revised but were not tested due the restricted amount of seed available (Table 4). Standard scarification treatments of 0.5 h or 3 h based on subgenus and seed size were not always effective in this study (Table 3). The six species with moderate or high germination were all categorized as soft or slightly hard; they responded moderately to both scarification treatments, and would need at most only a slightly longer scarification. *R. urticifolius* is small with hard seeds and a moderately thick seed coat that responded to 1 h H₂SO₄ scarification with good germination; but for species with large, very-hard seeds and a thick seed coat, even a 3 h scarification was probably suboptimal (Tables 3 and 4).

Our study of fresh *Rubus* seed found great diversity in germination requirements of 17 species in three subgenera and determined that seed-coat anatomy, especially seed-coat thickness and hardness were key factors in germination response (Wada and Reed, submitted for publication). Using the estimates in Table 4 as a guide, the optimum scarification for *Rubus* seeds should be determined by TZ testing following various lengths of scarification. Our seed sample did not include the very-thick, very-hard seeds (>0.4 mm) studied by Moore et al. (1974), but based on results from *R. chamaemorus* TZ testing it is likely that they would require 4–6 h of scarification.

Acknowledgements

Research was funded by the United States Department of Agriculture (USDA), Agricultural Research Service CRIS project

5358-21000-038-00D. USDA is an equal opportunity provider and employer. This research was part of a Ph.D. dissertation by Sugae Wada.

References

- Campbell, P.L., Erasmus, D.J., van Staden, J., 1988. Enhancing seed germination of sand blackberry. *HortScience* 23, 560–561.
- Clark, J.R., Eric, J.S., Hall, H.K., Finn, C.E., 2007. Blackberry breeding and genetics. In: Janick, J. (Ed.), *Plant Breeding*. John Wiley and Sons Inc., NJ, pp. 19–144.
- Clark, J.R., Moore, J.N., 1993. Longevity of *Rubus* seeds after long-term cold storage. *HortScience* 28 (9), 929–930.
- Daubney, H.A., 1996. *Brambles*. In: *Fruit Breeding II*. John Wiley and Sons, Inc., New York.
- Finn, C.E., 2008. *Rubus* spp., blackberry. In: Janick, J., Paull, R.E. (Eds.), *The Encyclopedia of Fruits and Nuts*. CABI, Cambridge, MA, pp. 348–351.
- Heit, C.E., 1967a. Propagation from seed: part 6 hardseededness. *Am. Nurseryman* 125, 1–5.
- Heit, C.E., 1967b. Propagation from seed: part 6 hardseededness; part 7 germinating 6 hardseeded groups. *Am. Nurseryman* 125, 1–9.
- Hummer, K., Peacock, D.N., 1994. Seed dimension and weight of selected *Rubus* species. *HortScience* 29, 1034–1036.
- Jennings, D.L., Tulloch, B.M., 1965. Studies on factors which promote germination of raspberry seed. *J. Exp. Bot.* 16, 329.
- Lasheen, A.M., Blackhurst, H.T., 1956. Biochemical changes associated with dormancy and after-ripening of blackberry seed. *Am. Soc. Hort. Sci.* 67, 331–340.
- Mian, M.A.R., Skirvin, R.M., Norton, M.A., Otterbacher, A.G., 1995. Drying interferes with germination of blackberry (*Rubus* sp.) seeds *in vitro*. *HortScience* 30, 124–126.
- Moore, J.N., Brown, G.R., Lundergan, C., 1974. Effect of duration of acid scarification on endocarp thickness and seedling emergence of blackberries. *HortScience* 9, 204–205.
- Peacock, D.N., Hummer, K., 1996. Pregermination studies with liquid nitrogen and sulfuric acid on several *Rubus* species. *HortScience* 31 (2), 238–239.
- Satomi, N., Naruhashi, N., 1971. Seeds of Japanese *Rubus*. I. Morphology. *Kanazawa Daigaku Rigakubu Fuzoku Shokubutsuen Nenpo* 4, 1–17.
- Wada, S., 2009. Evaluation of *Rubus* seed characteristics: seed coat morphology, anatomy, germination requirements and dormancy breaking. Ph.D. Dissertation, Horticulture, Oregon State University, Corvallis, p. 207.
- Wada, S., Reed, B.M., 2008. Morphological analysis of *Rubus* seed. *Acta Hort.* 782, 67–74.
- Wada, S., Reed, B.M. Standardizing germination protocols for diverse raspberry and blackberry species. *Sci. Hort.*, submitted for publication.
- Werker, E., Marbach, I., Mayer, A.M., 1979. Relation between the anatomy of the testa, water permeability and the presence of phenolics in the genus *Pisum*. *Ann. Bot.* 43, 765–771.
- Zasada, J.C., Tappeiner III, J.C., 2003. *Rubus* L. In: *The Woody Plant Seed Manual*. U.S.D.A. Forest Service, pp. 1629–1638.