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A temperate rhamnaceous species with a non-enclosing stone and without physical dormancy

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Abstract

The purpose of the present study was to investigate the dormancy break and germination requirements of seeds from the rhamnaceous vine *Berchemia scandens*. The fleshy fruit contains a two-locular stone with an endocarp described as 'bony, thickish'. Scarified and non-scarified stones increased by about 30–50% in mass during imbibition over a 24-h period. The endocarp of the stone does not completely enclose the seeds and a soft tissue region is present. This region is the primary area of water entrance to the seed, as shown by dye-tracking and by sealing it. Freshly matured and overwintered seeds of *B. scandens* germinated to low percentages at all temperatures during 2 weeks of incubation in light, and they germinated from moderate to high percentages during 12–14 weeks of incubation in light. While cold stratification had a relatively modest effect on the promotion of total germination across most temperatures assessed (if seeds were left for long enough), it had a somewhat stronger effect on germination rate. Cold-stratified seeds germinated equally well in light and darkness. The class of dormancy found in seeds of *B. scandens* would be physiological. The anatomy of the stones readily allows water imbibition, showing that seeds of *B. scandens* lack physical dormancy, an uncommon trait in Rhamnaceae.

Keywords: *Berchemia*, endocarp, imbibition test, physical dormancy, physiological dormancy, Rhamnaceae, stone

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Introduction

Of the 51 species in the plant family Rhamnaceae in which the dormancy class has been identified or inferred, 61% have physical dormancy (Baskin and Baskin, 1998, 2003a, b; Baskin *et al.*, 2000, 2007; Turner *et al.*, 2005; Huffman, 2006; Sautu *et al.*, 2006; Haines *et al.*, 2007; Saied *et al.*, 2008; Royal Tasmanian Botanical Gardens, 2009; Maraghni *et al.*, 2010). This type of dormancy is imposed by a water-impermeable layer of palisade or palisade-like cells in the seed or fruit coat. Dormancy break occurs with an opening in a specialized anatomical structure on the seed or fruit, allowing water entrance to the seed. For some members of the Rhamnaceae, the seed coat consists of a palisade layer of macrosclereid cells and the hilar fissure opens with heat (Corner, 1976; Orme and Leege, 1976; Keogh and Bannister, 1994; Baskin *et al.*, 2000; Foroughbakhch *et al.*, 2000; Doweld, 2001). In 22% of the 51 species, physiological dormancy is present in addition to physical dormancy, i.e. combinational dormancy. Once physical dormancy is broken, seeds still require some type of treatment, usually cold stratification in Rhamnaceae, for germination to proceed (Baskin and Baskin, 1998). Thus, a water impermeable seed/fruit coat is a common feature in the Rhamnaceae.

However, species with seeds having only physiological dormancy (12%) or being non-dormant (6%) are also reported in the Rhamnaceae (Mugasha and Msanga, 1987; Baskin and Baskin, 1998; Mattana *et al.*, 2009). Some species in this family with physiological dormancy or non-dormancy are congeneric with members having physical dormancy. In the genus *Ziziphus*, the semi-desert/desert species have physical dormancy while a semi-evergreen/evergreen rainforest species may be non-dormant (Baskin and Baskin, 1998; Saied *et al.*, 2008; Maraghni *et al.*, 2010). The pattern of some species having physical dormancy and others having non-dormancy, particularly those growing in tropical/subtropical regions, is a phenomenon seen in other plant families (Baskin and

Baskin, 1998; Jayasuriya *et al.*, 2010). Another pattern also emerges in the Rhamnaceae. In the genus *Rhamnus*, the sclerophyllous woodland species mostly have physical dormancy while the temperate deciduous forest/north-temperate montane/subalpine species have physiological dormancy (Baskin and Baskin, 1998; Mattana *et al.*, 2009).

Fruits of the woody, temperate vine *Berchemia scandens* (Hill) K. Koch (Rhamnaceae) are two-locular, one- or two-seeded, indehiscent, single-stoned drupes (Brizicky, 1964). The stone (endocarp) is described as 'bony, thickish', and the seed coat as 'membranaceous, thin', being adherent and partly adnate to the endocarp (Brizicky, 1964). The features of the endocarp and seed coat of *B. scandens* are identical to those reported for *Ziziphus*, a genus in which some members have physical dormancy (Brizicky, 1964; Baskin and Baskin, 1998). Physical dormancy along with physiological dormancy is reported in seeds of Kew's 'Difficult' seeds project species *B. discolor* – an African tree important for food, furniture, construction and dye (Hines and Eckman, 1993; Mgangamundo *et al.*, 2001; Royal Botanic Gardens Kew, 2012). 'Poor water permeability of [the] seed coat' hinders germination in the Asian *Berchemiella wilsonii* (Dang *et al.*, 2005), a genus closely related to *Berchemia* (Yilin and Schirarend, 2007). Therefore, we hypothesized that seeds of *B. scandens* would be physically dormant at maturity and perhaps also have a physiological component to dormancy. However, the description of the seed of *B. scandens* in Martin and Barkley (1961) is puzzling, stating that it has 'one end open'. A picture by them somewhat confirms this feature. If the seed does have an opening allowing water imbibition, then our hypothesis may be incorrect, at least with regards to physical dormancy.

The presence/absence of physical dormancy in *B. scandens* was examined by imbibition experiments on stones collected in late autumn when freshly matured and in late winter after overwintering on the vines. Dye-tracking was used to find the primary site of water entry to the seed, along with experimental blocking of this putative area during imbibition. In conjunction with understanding water entrance to the seed, gross and microscopic anatomical features were examined. Temperature and light requirements of germination were determined for seeds collected in late autumn and late winter. Using all of this information, we identified the class of dormancy (*sensu* Baskin and Baskin, 2004) in *B. scandens* seeds.

Materials and methods

Study species

Berchemia scandens grows in hammocks, rich well-drained woodlands, floodplain forests and swamps of south-eastern USA, southern Mexico and Guatemala (Brizicky, 1964; Godfrey and Wooten, 1981). The drupe is 5–8 mm long, bluish-black and ripens in late autumn; the mesocarp is fleshy and thin (Brizicky, 1964; Miller and Miller, 2005). Fruits persist on plants through most of winter and are eaten by a variety of bird and mammal species (Halls, 1977; Willson, 1993; Miller and Miller, 2005). The embryo fills a locule and endosperm is scarce (Martin, 1946).

Plant material

The experimental unit in our studies was a stone consisting of two (inseparable) locules; a locule contains a seed or it is empty. We use 'stone' when describing experimental protocols or in reference to both locules collectively (i.e. imbibition, anatomical and water entry studies). The term 'seed' is applied to the processes of dormancy and germination since the response (radicle emergence or not) and viability can be measured at the level of a locule.

Fruits of *B. scandens*, produced in the 2005 growing season, were collected from a population in Rutherford County, Tennessee (USA) on 2 December 2005, when they were freshly matured, and on 2 March 2006, after overwintering on vines. This population was found in a shrub thicket along a trail at the edge of a (mostly) redcedar (*Juniperus virginiana*) woodland. Fruits were also collected in November 2007 and in November 2011 from the same population for anatomical examinations. The exocarp and mesocarp (hereafter, pulp) were removed from the fruits, and then the stones were stored dry in a glass jar or in paper envelopes on a laboratory (21°C) shelf between collection and the initiation of studies.

Imbibition studies

Water uptake was investigated on 16 December 2005 and on 14 March 2006 for stones collected in December 2005 and March 2006, respectively. Scarification was achieved by removing a thin slice of the stone at its bottom end (opposite attachment of fruit stalk) with a single-edge razor blade. We took care to ensure that the slice slightly exposed the embryo but did minimal (or no) damage to it. Three replications each of 10 scarified and 10 non-scarified stones were placed on Whatman Number 1 filter paper moistened with distilled water in 6-cm-diameter Petri dishes on a laboratory bench. After 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0 and 24.0 h, stones were removed from the moist filter paper, blotted dry, weighed to the nearest 0.1 mg and returned to Petri dishes. Initial (t_o) mass was determined for stones that had been wetted for a brief period of time, blotted dry and weighed. Amount of water taken up was calculated as actual increase in

stone mass based on initial mass:

$$\%M_s = [(M_i - M_o)/M_o] \times 100, \quad (1)$$

where M_s = increase in mass of stones, M_i = mass of stones after a given interval of imbibition, and M_o = stone mass at t_o.

Anatomical examination

Hand sections of stones were made to observe gross anatomical features with a Leica (Model M80) stereomicroscope equipped with a Canon Rebel T3i camera. Vibratome (Vibratome 1500, St Louis, Missouri, USA) sections (25–30 μm) of stones were prepared, and the sections observed under an Olympus (Model BX 50) light microscope with photographs taken using a Canon EOS 30 D camera at the University of Kentucky (Lexington, Kentucky, USA). In addition, we examined stones at various times during stratification/incubation (see below) and during storage in the laboratory for any anatomical changes.

Determining putative water entry

Water uptake was investigated on 11 October 2006 for stones collected in December 2005. Stones were sealed by applying Elmer's super glue (Elmer's Products, Inc., Columbus, Ohio, USA) twice to their top (over the soft tissue region, see below) with a 24 h drying period between the two applications and between the last application and the start of the experiment. Three replications each of five sealed and five non-sealed stones were placed on Whatman Number 1 filter paper moistened with distilled water in 6-cm-diameter Petri dishes on the laboratory bench. The amount of water taken up over 24 h was recorded to follow imbibition (see above).

Twenty stones collected in March 2006 were placed in a Petri dish containing crystal violet solution and 20 other stones in a safranin solution. Five stones from each stain were removed at intervals of 1, 2, 3 and 4 weeks. After blotting stones to remove excess stain solution, longitudinal cuts were made through them using a razor blade. The staining pattern in relation to the stone's anatomy was observed under the stereomicroscope and recorded.

General germination procedures

Studies were done in temperature- and light-controlled incubators set at 12/12 h daily temperature regimes of 15/6, 20/10, 25/15, 30/15 and 35/20°C. These temperature regimes approximate mean daily maximum and minimum monthly air temperatures in Tennessee and adjacent states during: March and November, 15/6; April and October, 20/10; May, 25/15; June and September, 30/15; and July and August, 35/20°C (National Oceanic and Atmospheric Administration, 2003). The daily photoperiod was 14 h, extending from 1 h before the beginning of the high-temperature period to 1 h after the beginning of the low-temperature period. Another incubator was set at a constant 5°C and a 14-h photoperiod. This temperature was used for cold stratification since it is near-optimal for seeds of many species that require cold temperatures to come out of dormancy (Stokes, 1965; Nikolaeva, 1969). The light source in all incubators comprised 20 W cool white fluorescent tubes with a photon flux density (400–700 nm) at seed level from 48 $\mu mol\,m^{-2}\,s^{-1}$ (top shelf) to 72 $\mu mol\,m^{-2}\,s^{-1}$ (bottom shelf).

Stones collected in December 2005 and March 2006 were placed into incubators within 7 and 13 d, respectively, following collection. The substrate was white quartz sand, moistened with distilled water, in 6-cm-diameter plastic Petri dishes. Water was added to the dishes as needed to keep the substrate moist. Three replications of 25 stones (i.e. potentially 50 viable seeds) per dish were used in each treatment. Dishes containing seeds undergoing light treatments were wrapped with plastic film, and those in dark treatments were additionally wrapped in aluminium foil.

Protrusion of the radicle was the criterion for root emergence. Germination was judged at the seed level, i.e. if both seeds in a stone germinated then the data were recorded as two or if only one seed from a stone germinated then the data were recorded as one. At the end of each experiment, stones were dissected for examination. Locules from which seeds did not germinate were checked to see if they were empty or contained an embryo. Forceps were used to see if these embryos were firm and white, indicating that they were viable but dormant, or soft and brown, indicating they were non-viable. Tetrazolium tests confirmed our conclusions of viability based on observations.

Germination of freshly matured and overwintered stones

Stones used in this study were freshly matured (collected in December 2005) or had overwintered on the vine (collected in March 2006). Seeds were incubated in light or darkness at 15/6, 20/10, 25/15, 30/15 and 35/20°C with scoring for germination done every 2 weeks for a total of 14 (2005 collection) or 24 weeks (2006 collection). Seeds incubated in light for 14 or 24 weeks served as the controls for the cold stratification treatments (see below). Seeds from the 2005 collection were incubated in darkness and checked only at the end of 2 weeks. The 2006 collection was not tested for germination in darkness.

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Effects of cold stratification on dormancy break

Seeds (2005 collection) were cold stratified for 12 weeks in light and incubated at 15/6, 20/10, 25/15, 30/15 and 35/20°C for 2 weeks in light, and for 12 weeks in darkness and incubated for 2 weeks in darkness. In addition, one set of dishes ($n = 3$) was stratified in darkness and then opened before being moved to the incubation temperatures, to ascertain the amount of germination that occurred in darkness during the cold stratification period; these dishes were discarded after inspection. Seeds (2006 collection) were cold stratified for 6 or 12 weeks in light and incubated over the range of temperature regimes for up to 12 weeks in light, with seeds being checked for germination every 2 weeks.

Statistical analysis

Means and standard errors were calculated for the increase in mass of the stones during imbibition and for germination percentages. Germination percentages were determined on the basis of the number of viable seeds (once all stones were dissected and embryos examined), and they were arcsine square-root transformed for analyses. Repeated-measures analyses of variances (RMANOVAs) were used to test: (1) imbibition time as the within-subject effect and treatment (scarified versus non-scarified) and collection date (December versus March) as between-subject effects; (2) imbibition time as the within-subject effect and treatment (sealed versus non-sealed) as the between-subject effect; and (3) incubation time as the within-subject effect and seed condition (non-stratified versus 6-wk cold stratified versus 12-week cold stratified) and temperature regime (15/6–35/20°C) as the between-subject effects (SPSS, 2004). Greenhouse–Geisser corrected probabilities are reported for RMANOVA. A three-way analysis of variance (ANOVA) was used to examine the effects and interactions of seed condition (12-week cold stratified versus fresh versus control), temperature (15/6–35/20°C) and light regime (light versus dark) on germination percentages, and then means were compared by protected least significant difference tests (PLSDs, $P = 0.05$).

Results

Imbibition studies

Stones collected in December 2005 and March 2006 that were scarified, imbibed water, as well as those that were non-scarified (time, $P < 0.001$; all interactions with time, $P \geq 0.159$; treatment, $P = 0.743$; treatment × date, $P = 0.297$), but the percentage of water imbibed differed between the two collection dates (time × date, $P < 0.001$; date, $P = 0.013$). Scarified and non-scarified stones collected in December increased by 50 and 42%, respectively, in mass during imbibition over a 24-h period, and those collected in March by 27 and 33%, respectively (Fig. 1).

Anatomical examinations

The endocarp did not completely enclose the seeds (Fig. 2). A soft tissue region was present at the fruit stalk end of the stone and was separated by septa from the embryos. The soft tissue was cream coloured when fresh and was flush with (or protruded a little from) the endocarp. In the laboratory (dry conditions) and during stratification/incubation (moist conditions), the soft tissue receded and turned brown (laboratory) or black (incubation) over time. The septa under the soft tissue separated from the endocarp (forming a narrow crack) during dry or moist storage, especially near their base, and they could be easily removed from moist-stored stones by forceps. The radicle emerges through this crack (by pushing the septa aside) and then continues to elongate through the soft tissue region. We examined several widely separated

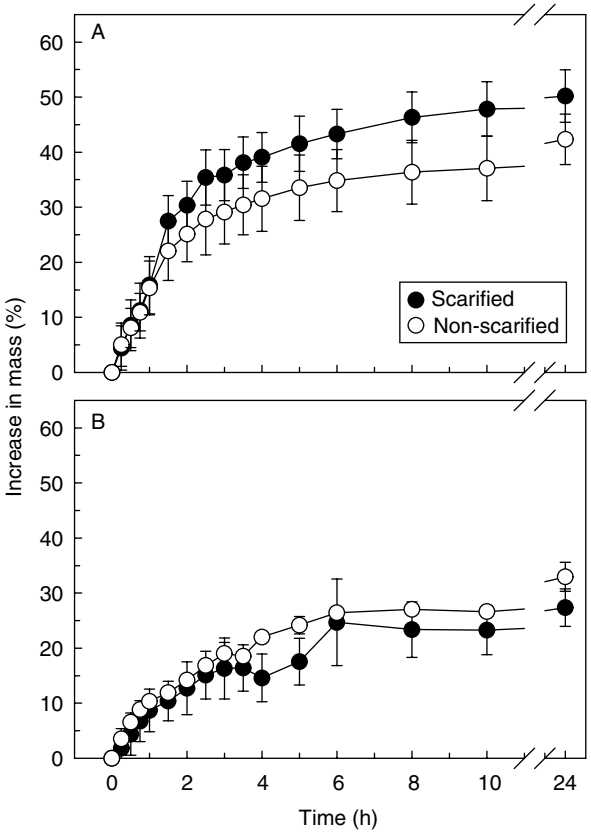

Figure 1. Mean (± SE) increase in mass of scarified and non-scarified stones of *Berchemia scandens* collected in December 2005 (A) and in March 2006 (B).

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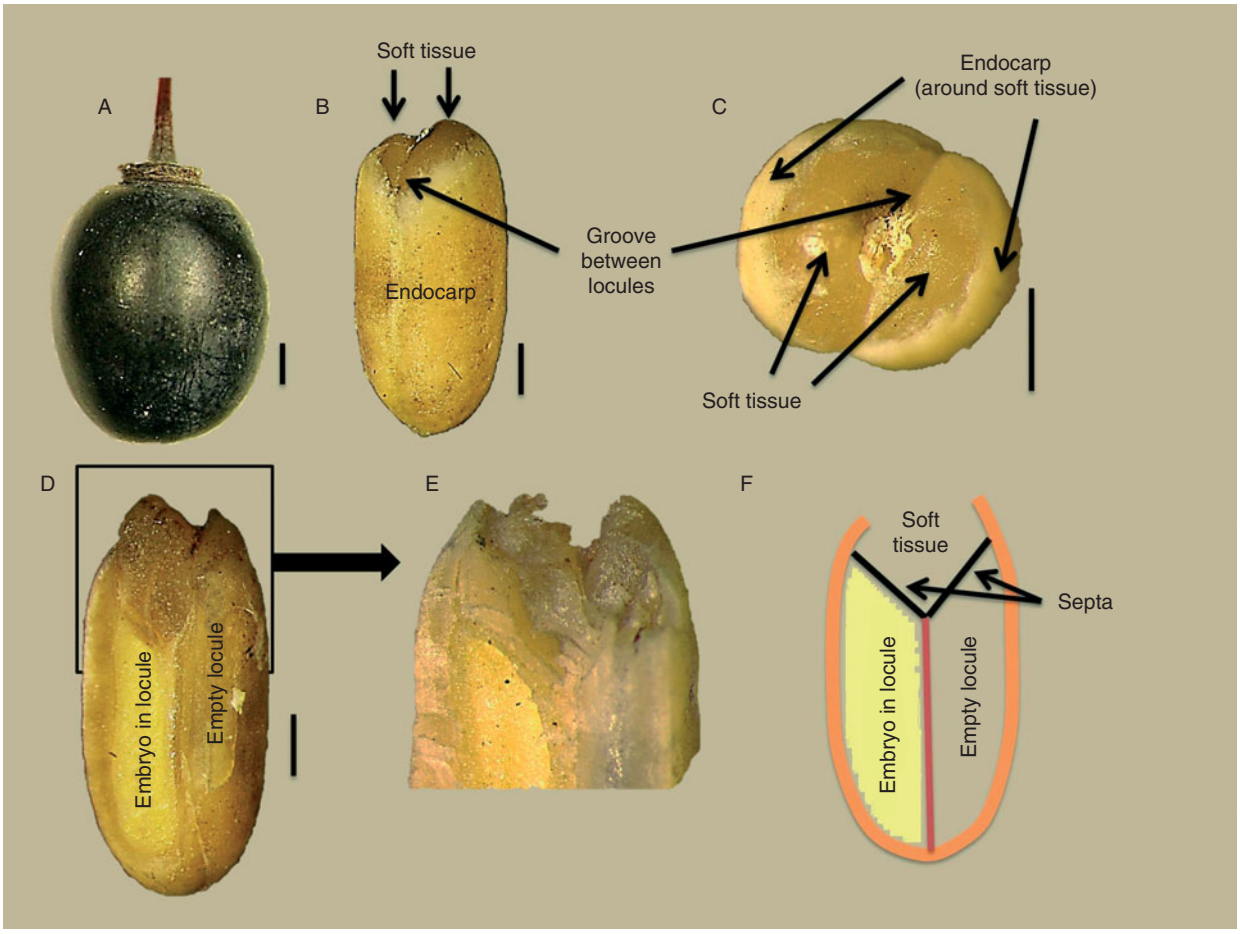

Figure 2. Gross anatomy of *Berchemia scandens*: (A) fruit with pulp intact and with a portion of the fruit stalk; (B) stone with pulp removed; (C) top view of soft tissue region; (D) longitudinal section through a stone; (E) enlarged longitudinal section through the soft tissue region showing its gradual deterioration over time (compare to panel D); and (F) schematic of longitudinal section through a stone as shown in panel (D) (orange line = endocarp and adnate seed coat, black lines = septa separating locules and soft tissue region, red line = partition between locules). A locule may or may not contain an embryo, and the locule with an embryo is usually larger than that without one. Vertical lines indicate scale (1 mm).

populations of this species in middle Tennessee at various times over several years, and the gross anatomy was always as reported here. The endocarp has 2–3 layers of relatively large, circular cells (sclereids) and 8–12 layers of small, elongated cells with cross walls (fibres) (Fig. 3).

Determining putative water entry

Sealing the top of the stone significantly reduced their water uptake (time, treatment and their interaction, $P \leq 0.007$). Stones in which the top was not sealed increased in mass by 44%, whereas those in which it was sealed increased by only 19% (Fig. 4).

Both stains entered the stone from the top via the soft tissue region, being obvious in the topmost part of a locule nearest the septa in week 1 and increasingly evident in weeks 2, 3 and 4 after placement of stones on the staining solutions. The stains progressively moved from the top of the stone, when by week 2 the upper 50–60% of the stone was violet or red, to the bottom of it, when by week 4 the entire inside of the locule was violet or red. We found no indication that crystal violet moved through the endocarp. However, staining of the internal endocarp (indicating passage of dye through it) was present with safranin in week 3 and particularly in week 4.

Germination of seeds

Condition, temperature and light had significant effects on the germination of seeds collected in December 2005 ($P \leq 0.045$). Within conditions, germination responses were similar between light regimes

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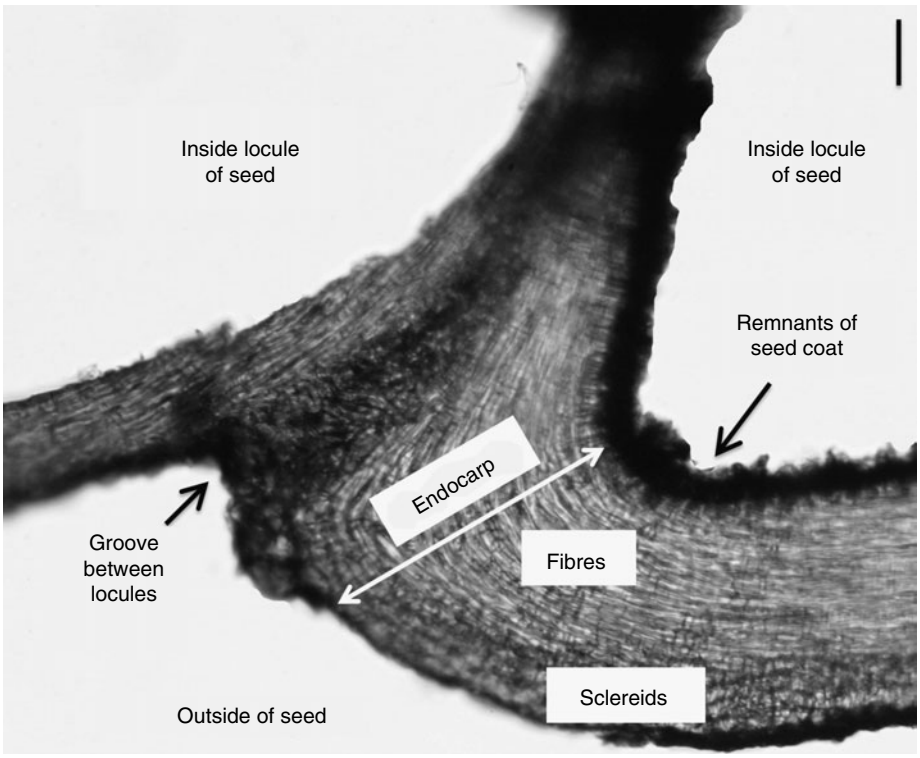

Figure 3. Transverse section through a stone of *Berchemia scandens* across a groove between locules (see Fig. 2). Scale bar (the vertical line in the upper right-hand corner of the figure) = 0.1 mm.

but dissimilar among temperatures (condition × light, light × temperature and condition × light × temperature, $P \geq 0.609$; condition × temperature, $P = 0.002$). Fresh seeds germinated between 0 and 30% during 2 or 4 weeks of incubation over the range of temperatures in light and darkness (Table 1). Seeds germinated to 35–74% in light and 14–71% in darkness during 2 weeks of incubation at all temperatures following 12 weeks of cold stratification in light or in darkness, respectively; non-stratified (control) seeds germinated to 54–88% during 14 weeks of incubation in light.

Germination responses of March 2006 seeds varied significantly over time and were highly dependent on their condition and temperature (time, condition and temperature factors, $P < 0.001$; all two- and three-way interactions, $P \leq 0.032$), but they were similar among conditions for each temperature (condition × temperature, $P = 0.085$). Germination increased over all temperatures with (1) increasing length of incubation; and (2) increasing amounts of cold stratification (Fig. 5). During 2 weeks of incubation, non-stratified seeds germinated to 0% at 15/6–20/10°C and to 18–35% at 25/15–35/20°C; 6-week cold-stratified seeds to 0% at 15/6°C and to 32–58% at 20/10–35/20°C; and 12-week cold-stratified seeds to 22–73% at 15/6–35/20°C. During 12 weeks of incubation, germination increased to 55–86, 69–90 and 82–90% over the range of temperatures for non-stratified, 6-week cold-stratified and 12-week cold-stratified seeds, respectively.

Discussion

Although the descriptions of the endocarp and seed coat of *B. scandens* are similar to those of other rhamnaceous species reported to have physical dormancy (Brizicky, 1964), scarified and non-scarified

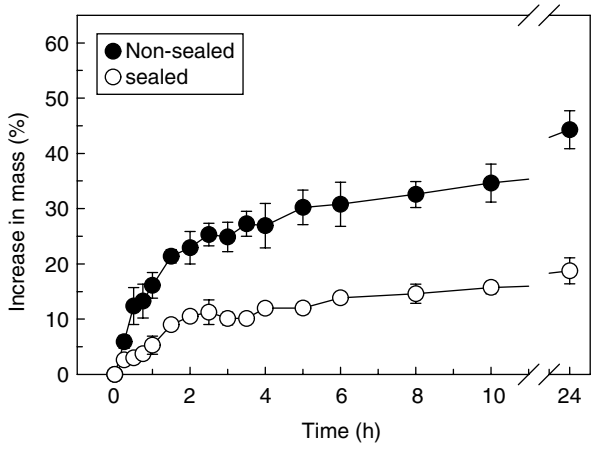

Figure 4. Mean (±SE) increase in mass of non-sealed and sealed stones of *Berchemia scandens*. Stones were sealed with super glue in the soft tissue region (see Fig. 2).

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Table 1. Germination percentages (mean ± SE) for seeds of *Berchemia scandens* (December 2005 collection)

	Light regime		Incubation temperature (°C)				
Condition	Str.	Inc.	15/6	20/10	25/15	30/15	35/20
Fresh (2 weeks inc.)		Light	0^{Aa}	0^{Aa}	0^{Aa}	5 ± 2^{ABb}	11 ± 5^{Ab}
		Dark	0^{Aa}	0^{Aa}	0^{Aa}	0^{Ba}	0^{Ba}
Fresh (4 weeks inc.)		Light	0^{Aa}	3 ± 2^{Aa}	14 ± 3^{Ab}	18 ± 5^{Ab}	30 ± 12^{ACb}
Control (14 weeks inc.)		Light	66 ± 5^{Ba}	75 ± 16^{Ba}	88 ± 3^{Ba}	77 ± 9^{Ca}	54 ± 12^{Ca}
Cold-stratified (12 weeks str. +2 weeks inc.)	Light	Light	35 ± 7^{Ca}	74 ± 10^{Ba}	67 ± 22^{Ba}	67 ± 8^{Ca}	49 ± 10^{Ca}
	Dark	Dark	14 ± 4^{Da}	68 ± 16^{Bb}	71 ± 5^{Bb}	56 ± 18^{Cb}	40 ± 4^{Cab}

Non-stratified seeds were incubated (inc.) in light or darkness for 2 or 4 weeks (fresh) and in light for 14 weeks (control). Cold-stratified seeds were stratified (str.) and incubated in light or stratified and incubated in darkness. Stratification occurred at 5°C for 12 weeks, and incubation followed over the range of temperature regimes for 2 weeks. No seeds germinated in light or darkness during the cold stratification period. Values with different upper-case letters within columns or lower-case letters within rows are significantly different (protected least significant difference test, $P = 0.05$).

stones of *B. scandens* increased in mass by 30–50% whether they were collected in December when freshly matured or in March when overwintered on the vine (Fig. 1). In *B. scandens* stones, the endocarp does not enclose the seeds and a soft tissue region is present (Fig. 2). This soft tissue region gradually deteriorates over time and the stone appears to have 'one end open', as described in Martin and Barkley (1961). We are unaware of any other fruits with similar anatomy, with the exception of other species of *Berchemia*. The gross anatomical features of *B. scandens* (with a recessed soft region) are similar to: the African trees *B. discolor* and *B. zeyheri* (TopTropicals, 2012) and the Asian shrubs *B. berchemiifolia* (= *Berchemiella berchemiifolia*) and *B. floribunda* (Lee *et al.*, 2010).

For *B. scandens*, water enters the seed primarily via the soft tissue region, as evidenced by (1) dye tracking and (2) sealing it (Fig. 4). A limited amount of water enters through the endocarp. The composition of the endocarp for *B. scandens* stones (Fig. 3) is similar to that for stones of other species in which the entire seed is enclosed by endocarp and presumed to be impermeable but was not once tested for imbibition. For example, in fruits of *Symphoricarpos orbiculatus* (Caprifoliaceae) the dispersal unit consists of pulp (exocarp and mesocarp) and the stone (fibrous endocarp and seed). However, non-scarified and scarified stones readily imbibed water (Hidayati *et al.*, 2001). Although stones of *B. scandens* (and *S. orbiculatus*) are described as 'hard' or impermeable, they lack the characteristic anatomy of physically dormant seeds, e.g. a water impermeable palisade layer (Baskin and Baskin, 1998).

Freshly matured and overwintered seeds of *B. scandens* germinated to low percentages at all temperatures during 2 weeks of incubation and they germinated from moderate to high percentages during 12–14 weeks of incubation (Table 1, Fig. 5). Compared to non-stratified (control) seeds, cold stratification had a relatively modest effect on the promotion of total germination across most temperatures assessed (if seeds were left long enough). On the other hand, cold stratification had a stronger effect on germination rate (compare lines across time and temperature in Fig. 5). In a forest, Shelton and Cain (2002) found that *B. scandens* seeds (probably stones) buried under the leaf litter and fermentation layers of the floor in November 1996 germinated to high percentages when removed in April and incubated at 21°C. These buried seeds had been cold stratified for about 5 months during winter in the field, suggesting the beneficial effect of this process on dormancy break. In our work, seeds collected in December 2005 germinated up to 30% during 4 weeks of incubation in light (Table 1) and those collected in March 2006 (after overwintering on vines) germinated up to about 70% (Fig. 5). These results show that cold stratification can also take place on the plant during winter.

From an ecological perspective, seeds of *B. scandens* would probably be unable to germinate in nature when dispersed from late autumn to early spring. No fresh seeds germinated at temperatures corresponding to these seasons (≤15/6°C) during 2 weeks of incubation (Table 1) and no overwintered seeds did so during 6 weeks of incubation (Fig. 5). If seeds were dispersed during this time period and received cold stratification on the forest floor or on the plant, we predict that most of them would germinate in early spring once temperatures warmed to ≥15/6°C, which approximates temperatures in March. Moreover, the pulp would probably need to be removed (via decay or frugivores) for effective dormancy break and germination to occur, as has been found for other fleshy-fruited shrubs (Meyer and Witmer, 1998; Karlsson *et al.*, 2005). The longevity and viability of seeds on vines past March are unknown, but many seeds were present on the vines at the time of collection in March 2006. Seeds dispersed between approximately April and October, when temperatures would be ≥20/10°C, would probably germinate (if pulp was removed), since

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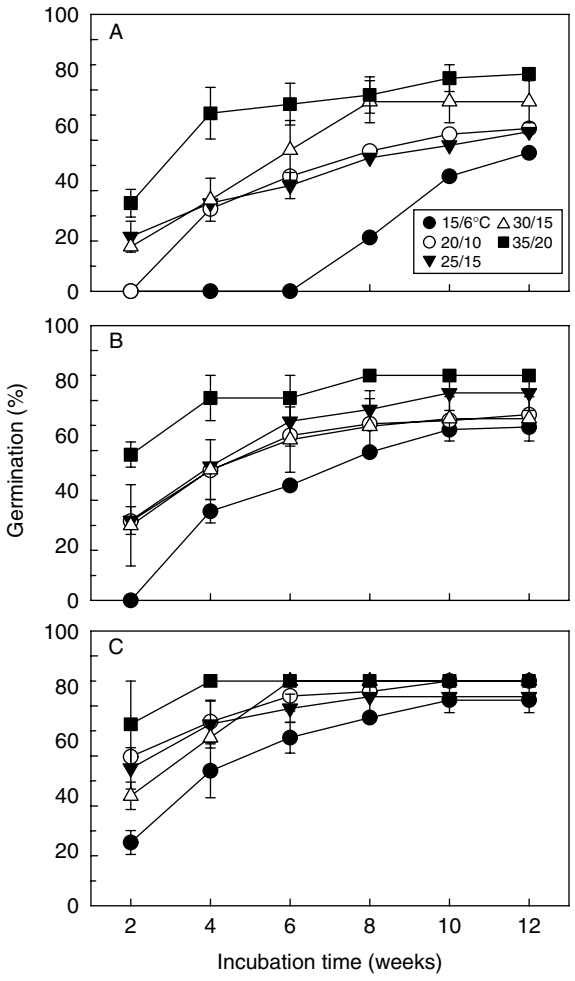

Figure 5. Mean (±SE, SE shown if ≥5) cumulative germination percentages of overwintered *Berchemia scandens* seeds (March 2006 collection) that were: (A) non-stratified (control) and incubated for 12 weeks over the range of temperatures; (B) cold stratified for 6 weeks and incubated for 12 weeks; and (C) cold stratified for 12 weeks and incubated for 12 weeks. Seeds were exposed to a 14-h photoperiod in each treatment.

seeds in the laboratory studies germinated from moderate to high percentages at these temperatures without cold stratification (Fig. 5A).

In Shelton and Cain's (2002) study of buried *B. scandens* seeds, only 1–3% were viable after burial for 3 years, indicating a low potential for carry-over. Consistent with their observations, we found that seeds of this species germinated equally well in light and darkness following cold stratification (Table 1) and thus they could germinate during burial. Indeed, germinating seeds were observed in bags removed in March from the forest floor after 2 years of storage (Shelton and Cain, 2002). Apparently, the majority of seeds from this species germinate during the first spring following dispersal and they have a limited capacity to form a (long-lived) persistent soil seed bank (*sensu* Walck *et al.*, 2005).

Five classes of seed dormancy were recognized by Baskin and Baskin (2004). Two classes of dormancy, morphological and morphophysiological, include seeds in which the embryo is not differentiated or, if it is differentiated, the embryo is underdeveloped (small relative to the amount of endosperm). In contrast, embryos are differentiated and fully developed in seeds with (only) physiological dormancy, physical dormancy and combinational dormancy. The seed/fruit coat is permeable to water in seeds with physiological dormancy and not permeable in those with physical dormancy or combinational dormancy. Morphological and morphophysiological dormancies can be ruled out in *B. scandens* since the embryos completely fill the seed and endosperm is scarce (Martin, 1946; Brizicky, 1964). In addition, the stones of this species readily imbibed water and so lack physical or combinational dormancies. Since cold stratification improved germination percentages and rates of *B. scandens* seeds, we conclude that they have a modest degree of physiological dormancy. This class of dormancy is caused by a physiological inhibiting mechanism, and structures that cover the embryo may also play a role in preventing germination (Baskin and Baskin, 1998). For *B. scandens* seeds, there is very little mechanical resistance to radicle emergence, given that the septa covering them are easily removed (especially under moist conditions).

Seed dormancy break and germination have, to the best of our knowledge, only been investigated in one other *Berchemia* species, with somewhat contradictory results. Seeds of the African *B. discolor*, a species of semi-arid grassland and savanna, have been reported to have combinational (physical + physiological) dormancy in some cases (Royal Botanic Gardens Kew, 2012). Hines and Eckman (1993) suggested scarifying the seeds or immersing them in hot water and then allowing them to cool for 12 h as one way to overcome dormancy. On the other hand, Mgangamundo *et al.* (2001) found that seed coat impermeability was not the only cause for prolonged and low germination for *B. discolor* and recommended that the seeds be chopped at the radicle end followed by soaking in cold water and drying in the sun for 24 h as a method to promote germination. In both cases, the treatments used would have been beneficial for breaking physical and physiological dormancies in *B. discolor* seeds. In contrast, the results reported by Orwa *et al.* (2009) suggest that the *B. discolor* seeds they assessed in their study were non-dormant, as germination from sown fresh (untreated) seeds in trays filled with a mixture of river sand and compost was 80–100%. Given that the seeds of *B. discolor* superficially resemble those of *B. scandens* (TopTropicals, 2012), it would be interesting to explore imbibition and anatomy in *B. discolor* seeds

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in light of the fact that congeneric species of Rhamnaceae often differ in their dormancy class among biomes.

Of all the most closely related genera to *Berchemia* (Brizicky, 1964; Richardson *et al.*, 2000), information on dormancy and germination is only available on *Berchemiella*. Poor water permeability of the seed coat was reported in seeds of *Berchemiella wilsonii* var. *pubipetiolata* (Dang *et al.*, 2005). However, seeds treated with 98% sulphuric acid and those without treatment absorbed approximately 2.1 and 1.7 g, respectively, of water during 96 h. Moreover, germination was markedly improved when seeds were soaked in gibberellic acid (GA_3) for 24 h. We conclude that *B. wilsoniii* seeds have physiological dormancy, since (1) acid-scarified and non-scarified seeds absorbed similar amounts of water; and (2) GA_3 improved germination, which is a common response of seeds with non-deep physiological dormancy (Baskin and Baskin, 1998).

Previously, seeds from some members of the genus *Rhamnus* were reported to have only physiological dormancy (Baskin and Baskin, 1998; Mattana *et al.*, 2009). Thus, our report of *B. scandens* seeds having only physiological dormancy represents the first confirmation of the presence of this dormancy class in a genus of Rhamnaceae other than *Rhamnus*. In contrast to *Rhamnus*, stones of *B. scandens* are not completely enclosed by the endocarp and contain a soft tissue region that readily allows entrance of water and allows emergence of the root. To the best of our knowledge, the anatomical features of *B. scandens* stones have not been reported heretofore in any other plant.

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