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Seasonal influence on dormancy alleviation in *Dodonaea viscosa* (Sapindaceae) seeds

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Abstract

Seeds of *Dodonaea viscosa* (Sapindaceae) have a water-impermeable seed coat, i.e. physical dormancy (PY). Although mechanical scarification, dry heat, sulphuric acid and hot water treatment make seeds permeable under laboratory conditions, the mechanisms by which dormancy is alleviated in natural environments have not yet been understood completely. The present investigation aims to understand the pattern of dormancy alleviation in *D. viscosa* seeds using an artificial burial approach for 2 years. Freshly collected seeds held in hydrated soil at 10/20°C, 15/20°C, 15/30°C, 20/35°C and 25°C for 32 weeks germinated to less than 15%, irrespective of storage temperature. Dry storage of seeds at 15, 20, 25 and 30°C for 1 year did not break dormancy. Hot water treatment at 80 and 90°C for 30 s broke dormancy in 90% of the seeds. On the other hand, burying seeds at a depth of 3–5 cm in the natural environment for 2 years increased germination from 7 to 71%. In particular, seeds exhumed after summer in both years showed a significant increase in germination percentage ($P < 0.05$). However, seeds buried after summer did not germinate to a higher percentage when exhumed prior to summer. We suggest that a high summer temperature, rising above 60°C in the top soil layer of the tropics, is a likely factor breaking dormancy. Most seeds germinated during burial, which indicates that light is not a cue for germination. We conclude that germination of *D. viscosa* following summer is an adaptive mechanism to tolerate summer droughts, which are common in the dry tropics.

Keywords: artificial burial, *Dodonaea viscosa*, germination, natural adaptation, physical dormancy, seed ecology

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Introduction

Seeds of *Dodonaea viscosa* (Sapindaceae) have a water-impermeable seed coat, i.e. physical dormancy, PY (Burrows, 1995; Baskin *et al.*, 2004; Phartyal *et al.*, 2005; Benítez-Rodríguez *et al.*, 2013), although some studies have claimed that the seeds of this species are non-dormant (see Baskin *et al.*, 2004). The Sapindaceae is one of 17 angiosperm families that produce seeds with PY, and this form of dormancy has not been reported in any gymnosperms (Baskin and Baskin, 1998; Baskin *et al.*, 2000; Cook *et al.*, 2008; Jayasuriya *et al.*, 2008b; Turner *et al.*, 2009). Genera of Sapindaceae, including *Dodonaea* (Burrows, 1995; Baskin *et al.*, 2004; Phartyal *et al.*, 2005; Benítez-Rodríguez *et al.*, 2013; Nasr *et al.*, 2013), *Distichostemon* (Cook *et al.*, 2008) and *Cardiospermum* (Johnston *et al.*, 1979) have seeds that germinate once the seed coat becomes permeable and, therefore, possess only PY. In some Sapindaceae genera, such as *Diplopeltis* (Turner *et al.*, 2006) and *Koelreuteria* (Garner, 1979; Rehman and Park, 2000), in addition to the seed coat being impermeable to water, the embryo exhibits physiological dormancy (PD). Thus, these species have combinational dormancy, i.e. PY + PD. However, since the seeds of *D. viscosa* germinate when the coat becomes permeable to water without any additional treatment, the embryo of this species does not possess PD, thereby confirming the presence of only PY (Baskin *et al.*, 2004; Phartyal *et al.*, 2005; Benítez-Rodríguez *et al.*, 2013).

In most species with PY, the seed coat becomes impermeable to water only during the final stage of maturation drying, due to the palisade layer of the lignified Malphigian cells (Rolston, 1978; Baskin and Baskin, 1998; Baskin *et al.*, 2000). However, the impermeable nature of the seed coat is maintained until a specific dormancy breaking cue opens a small specialized anatomical structure (water gap) which serves as an entry point for water. Numerous treatments, including sulphuric acid, mechanical scarification, dipping in boiling water, dry heat, high temperatures, fire, drying, freeze–thaw cycles and passage through the digestive tracts of animals, can

break PY in seeds (Rolston, 1978; Baskin and Baskin, 1998; Baskin *et al.*, 2000). While sulphuric acid, mechanical scarification, nicking of the seed coat and hot water treatment can effectively break dormancy in *D. viscosa* seeds (Burrows, 1995; da Rosa and Ferreira, 2001; Baskin *et al.*, 2004; Phartyal *et al.*, 2005; Benítez-Rodríguez *et al.*, 2013; Nasr *et al.*, 2013), such treatments have little, if any, ecological value in alleviating dormancy. In the field, seeds mostly break PY by experiencing a brief period of chilling temperatures or fluctuating temperatures in temperate zones (Baskin and Baskin, 1998; Van Assche *et al.*, 2003), or warm soil temperatures in tropical locations (Probert, 2000; Turner *et al.*, 2009). Ecologically, dormancy breaking cues must be driven primarily by the combination of seasonal temperature changes, because factors such as fire or passing through the digestive tracts of animals are sporadic; therefore a seed may not undergo these mechanisms of dormancy breaking for long periods of time.

In Australia, seeds of many Sapindaceae species become permeable to water in nature while experiencing warm and moist conditions during summer (Cook *et al.*, 2008; Turner *et al.*, 2009). In *Dodonaea hackettiana*, summer temperatures effectively broke dormancy and, consequently, improved total germination (Cook *et al.*, 2008). According to Turner *et al.* (2009), high temperatures open a specialized 'water gap' present in *Dodonaea* seeds, thereby allowing the seeds to imbibe water and germinate. However, detailed information on how dormancy is alleviated in other adapted species is lacking, although the genus *Dodonaea* has a widespread geographical distribution from tropical to temperate regions (Liu and Noshiro, 2003; Baskin *et al.*, 2004; Harrington and Gadek, 2009). Furthermore, to the best of our knowledge, long-term burial experiments with Sapindaceae species having PY do not exist and such information seems to be highly restricted to species of Fabaceae, Geraniaceae and Malvaceae (Baskin and Baskin, 1998; Van Assche *et al.*, 2003; Van Assche and Vandelook, 2006; Hu *et al.*, 2009). As a result, we were interested in understanding the dormancy breaking mechanisms of *D. viscosa* in its natural environment. Thus, our study has two main objectives: (1) to determine if *D. viscosa* from southern India produces dormant seeds; and (2) to document dormancy loss in *D. viscosa* by incorporating seeds in an artificial burial experiment.

Materials and methods

Seed collection

Seeds of *D. viscosa* were collected from 10–15 plants growing on roadsides in Anaikati, Tamil Nadu, India (11°10′ N, 76°74′ E) on 22 December 2010 and on 3 and 7 January 2011. The collection site is a tree-dominated landscape that lies adjacent to the Western Ghats. The climate is warm with approximately more than 150 days with soil temperatures above 50°C (see results). Rainfall is mostly brought by the south-west monsoon during July to October, sometimes lasting until November, and the north-east monsoon during October and November, rarely continuing during December (Agro Climate Research Centre, Tamil Nadu Agricultural University, Coimbatore, India). Seeds collected from individual plants were grouped into one lot, mixed thoroughly and stored in jute bags at room temperature (25–30°C; *c.* 50–60% RH) until used in the experiments. The number of days between first seed collection and the inception of experiments was less than 1 month.

Moisture content measurement

Moisture content of the freshly collected seeds was determined by drying four replicates of 100 seeds each at 103°C for 17 h and weighing seeds before and after drying (International Seed Testing Association, 2009). Moisture content is expressed as percentage of fresh weight (mean ± SD).

Germination test

Four replicates of 25 seeds each were germinated in Petri dishes containing 1% agar-water. Seeds were incubated at an alternating temperature of 20/30°C at a 12 h photoperiod. Light was provided during the warm phase by cool white fluorescent tubes at approximately 40 $\mu mol\, m^{-2}\, s^{-1}$, 400–700 nm. Radicle emergence was the criterion for germination, and germination counts were made daily for 21 d. Total number of seeds germinated is presented as a percentage (mean ± SD). Following the different dormancy-breaking treatments, unless stated otherwise, seeds were tested for germination at 20/30°C with the same light conditions as described here.

Dormancy breaking

In order to break dormancy, seeds were both mechanically scarified (by a razor blade) and dipped in hot water for 5 s (Phartyal *et al.*, 2005). In all cases, the temperature of the hot water was 90 ± 3°C. In a separate experiment, the importance of hot-water temperature in breaking dormancy was evaluated by dipping seeds in water at 40, 50, 60, 70, 80 and 90°C for 30 s. Three replicates of 25 seeds were used for each temperature. In addition, freshly collected seeds were stored dry in empty Petri dishes under natural light at four different temperatures: 15, 20, 25, 30°C.

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Three replicates of 50 seeds each were used. Freshly collected seeds were sown on natural soil in 9-cm Petri dishes. The soil was kept hydrated by adding water whenever required. The Petri dishes were incubated in light (12 h; 30 μmol m^{-2} s^{-1}, 400–700 nm) at 10/20°C, 15/20°C, 15/30°C, 20/35°C and 25°C (constant temperature). Four replicates of 25 seeds each were placed at each temperature, and germination was scored weekly for 32 weeks.

Seed imbibition

Three replicates of 20 seeds were used to monitor the imbibition of water in mechanically scarified, hot-water-treated and control (without any treatment) seeds. The seeds were placed in Petri dishes on wet filter paper and kept under laboratory conditions. The increase in mass was calculated by weighing the seed mass with a micro-balance at hourly intervals, after drying the surface of the seed between soft tissue towels. The percentage increase in seed mass of seeds was determined following Baskin *et al.* (2004).

Seed burial experiment

One hundred freshly collected seeds were placed in each of 16 nylon mesh bags and buried at a soil depth of 3–5 cm. Four bags were placed in each of four plastic trays (15 cm × 8 cm × 4 cm) which contained drainage holes that facilitated water movement during burial. Before use, trays were filled with natural soil and covered with a loose lid (clipped), to prevent any accidental damage, e.g. predation. Thus, although we intentionally prevented any accidental damage that might be associated with seed dispersal, this experimental set-up did not affect the conditions seeds would experience in nature, e.g. temperature, soil moisture, etc. The trays were placed at four different locations within the experimental site (i.e. seed collection location) on 10 February 2011. The site was fenced before starting the burial experiments. After 6 months (25 August 2011), 12 months (21 February 2012), 18 months (30 August 2012) and 24 months (28 February 2013) the bags (one tray each time) were exhumed and the contents were spread on a laboratory bench. Seeds germinated in the soil were counted and the remaining seeds were incubated at 20/30°C for germination, as a replicate of (100 − number of seeds germinated in soil) × 4. Germinated seeds were counted and removed on a daily basis. After 3 weeks the remaining non-germinated seeds were dipped in hot water (5 s) and returned to 20/30°C.

In preliminary trials, germination during burial obscured when seeds had germinated during burial periods of longer than 6 months. To obviate this confounding issue, we buried an additional 400 seeds, placed in one bag, at the time when the first batch of seeds buried in February 2011 was exhumed, i.e. 25 August 2011. These seeds were stored dry in Petri dishes at room temperature between collection (January 2011) and burial in August 2011. Prior to burial, the germinating ability of these seeds was tested, as described above, from a subset of stored seeds, and at this time 9 ± 2.5% seeds germinated. The seeds buried in August were exhumed in February 2012 and germinated separately.

Soil temperature measurement

Soil temperature during seed burial was recorded at 1 h intervals using a data logger fixed firmly to the ground with a thick screw clamp at 1–2 cm below the soil surface, which measured the soil temperature in the surroundings of buried seeds at a depth of 3–5 cm.

Statistical analysis

The percentages of seeds germinating after various periods of burial were compared using analysis of variance (ANOVA). To improve the normality, data were arcsin transformed before testing for statistical significance, but the actual germination percentages are reported. We used the Turkey HSD *post-hoc* test ($\alpha = 0.05$) to check the significance between groups.

Results

Germination and moisture content

Moisture content of the freshly collected seeds was 7.5 ± 0.9%. Seeds at the time of collection only germinated to 7 ± 1.3% without any pre-treatment. However, hot-water treatment and mechanical scarification increased germination to 91 ± 2.2% and 95 ± 1.0%, respectively (Fig. 1). In general, mechanically scarified seeds germinated faster than the hot-water treated and control seeds. After 24 h the mass of mechanically scarified seeds increased to 99 ± 2%, but the mass of the seeds treated with hot water and control seeds only increased to 34 ± 4% and 8 ± 2%, respectively (data not shown; see Baskin *et al.*, 2004; Phartyal *et al.*, 2005).

Dormancy breaking

Seeds stored dry at 15, 20, 25 and 30°C for 1 year only germinated to <15%, when incubated at 25°C and 15/30°C for 21 d at the end of storage (Fig. 2). However, hot-water treatment (90°C) for 30 s increased germination percentage to >90% in seeds stored at all four temperatures.

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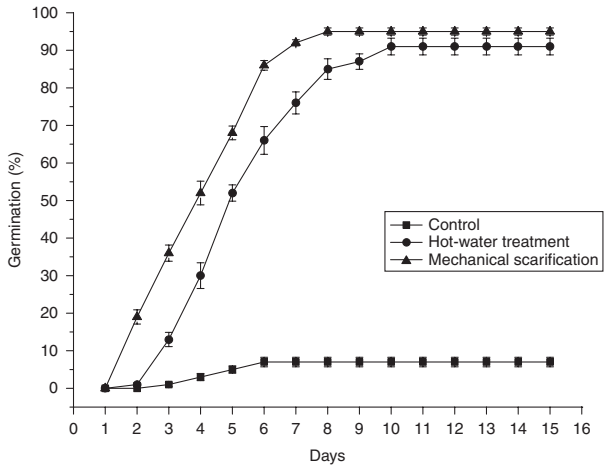

Figure 1. Germination percentage of untreated (control), mechanically scarified and hot-water treated *D. viscosa* seeds at 20/30°C for 15 d. Error bars indicate standard deviation.

Germination of seeds stored at 10/20°C, 15/20°C, 15/30°C, 20/35°C and 25°C ranged from 5 ± 1.5% at 10/20°C to 13 ± 3.2% at 15/30°C after 32 weeks. Seeds at 15/20°C and 20/35°C germinated to 7 ± 2.1% and 12 ± 2.7% respectively. Seeds held at a constant temperature of 25°C showed a final germination of 9 ± 1.5%. At all the temperatures germination occurred during the first 3 weeks of storage.

The temperature of the water had a significant effect on dormancy breaking of *D. viscosa* seeds ($F_{(5,12)} = 23.98$, $P < 0.05$; Fig. 3). Dormancy was not broken to a significant level in seeds unless the temperature of the water was between 70°C and 90°C ($P < 0.05$; Fig. 3). However, germination after dipping seeds in water at 70°C was slow, with most seeds germinating only during the second week. Dipping seeds in water at 80 and 90°C effectively broke dormancy in 88 and 96% of seeds, respectively ($P < 0.05$), with most seeds completing germination within 2 weeks.

Soil temperature

Soil temperature measurements during the 2-year burial period showed that the months between April and July were warmer, with May the warmest (Fig. 4). Temperatures ranged from 15°C in February 2011, March 2011 and December 2012 to 66°C in May 2012. In total, there were 170 and 148 d in 2011 and 2012, respectively, with temperatures rising above 50°C (Fig. 4). During the burial period, soil temperatures never dropped below 15°C (Fig. 4). Higher temperature fluctuations were observed during the warmer months of the year compared to a narrow fluctuation range of 10°C between September and February.

Seed burial and germination

In contrast to low germination at the time of collection, seeds buried in their natural environment showed improved germination with time (Fig. 4). There was a significant time × dormancy loss effect observed during burial, $F_{(4,15)} = 19.96$, $P < 0.05$. Seeds retrieved after 6 months in August significantly (but marginally) improved germination as compared to the control ($P < 0.05$; Fig. 4). Within 6 months of burial, germination percentage increased to 37 ± 12.3%, as compared to 7% at the time of burial. Although seeds exhumed after 1 year showed higher germination percentages (47%) as compared to August-retrieved seeds (37%), the effect was not statistically significant ($P > 0.05$). However, seeds exhumed on 2 August (2012) showed a significant increase in germination ($P < 0.05$) and by this time 73 ± 11.7% of the buried seeds had germinated. Percentage germination of seeds buried for 2 years was only 71 ± 13.2% ($P > 0.05$). Of the 400 seeds buried in August 2011, only 22 ± 9.5% of the seeds had germinated after 6 months in soil. Most

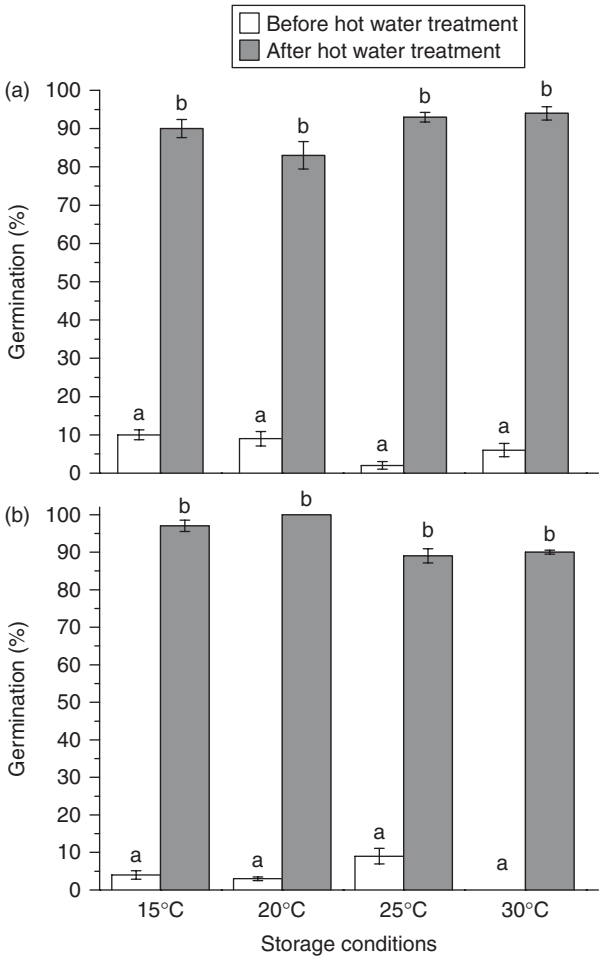

Figure 2. Germination percentage of *D. viscosa* seeds stored for 1 year at four different temperatures and germinated at (a) 25°C constant and (b) 30/15°C before and after dipping in hot water.

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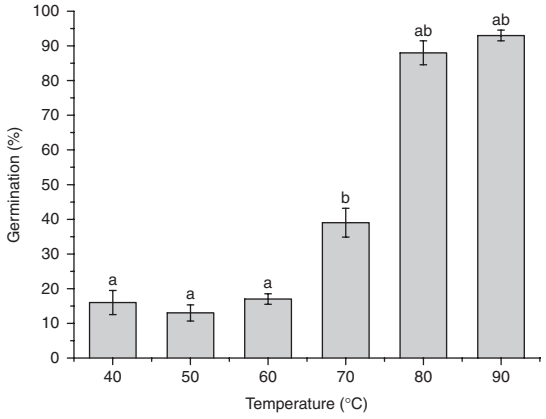

Figure 3. Germination percentages (mean ± SD) of *D. viscosa* seeds dipped in hot water at different temperatures for 30 s and incubated at 20/30°C for 3 weeks. Temperatures at which significant changes ($P < 0.05$) occurred are marked with different lower-case letters.

of the seeds germinated during burial and only very few did so when incubated at 30/20°C for 3 weeks. Seeds that remained non-imbibed at the end of the germination test germinated to nearly 90% after dipping in hot water (Fig. 4).

Discussion

The experiments confirm that seeds of *D. viscosa* from southern India have PY and only germinate after dormancy is broken. Similar results were reported for *D. viscosa* occurring in Australia (Hodgkinson and Oxley, 1990), Brazil (da Rosa and Ferreira, 2001 cited by Baskin *et al.*, 2004), Hawaii (Baskin *et al.*, 2004), Mexico (Gonzalez Kladiano and Camacho Morfin, 1994 cited by Baskin *et al.*, 2004; Benítez-Rodríguez *et al.*, 2013), northern India (Phartyal *et al.*, 2005) and New Zealand (Burrows, 1995). This study therefore adds additional support to the earlier suggestion by Baskin *et al.* (2004) and Phartyal *et al.* (2005) that: (1) seeds of *D. viscosa* from all geographical locations must be dormant; and (2) studies claiming that seeds of *D. viscosa* are non-dormant must have collected seeds before they attained full maturity.

It is reasonable to speculate that a small proportion of *D. viscosa* seeds at the time of maturity may have been non-dormant and germinated without any dormancy-breaking treatments (Fig. 1), as reported previously for species within the Sapindaceae (Burrows, 1995; Cook *et al.*, 2008) and other families with PY, e.g. Fabaceae (Morrison *et al.*, 1992; Moreira *et al.*, 2010; Hu *et al.*, 2013) and Cistaceae (Moreira *et al.*, 2010).

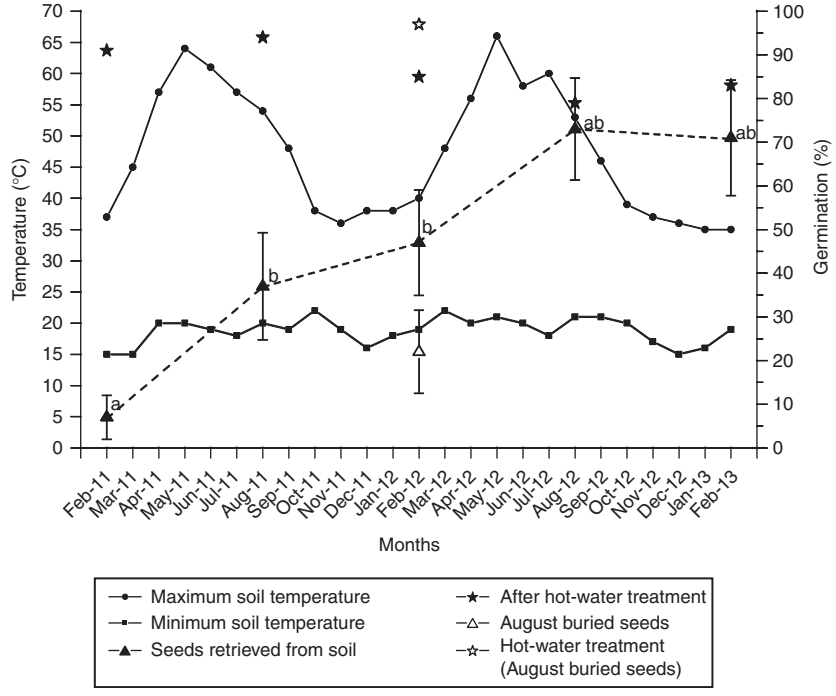

Figure 4. Maximum and minimum soil temperature in °C recorded at 3–5 cm below the soil surface at the study site between February 2011 and January 2013. Dormancy loss corresponding to the seasonal temperature fluctuation is presented as germination percentage (mean ± SD) of buried seeds exhumed every 6 months and tested at 20/30°C, for 2 years between February 2011 and February 2013. The percentage of seeds that remained dormant and germinated after hot-water treatment is indicated by asterisks. Error bars represent standard deviation of the mean. Data points with different lower-case letters represent statistically significant differences in germination percentages.

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However, this argument is likely to be affected by the seed collection environment. For example, although only less than 2% of the seeds from Hawaii (Baskin *et al.*, 2004), Mexico (Benítez-Rodríguez *et al.*, 2013) and New Zealand (Burrows, 1995) germinated at the time of collection, Phartyal *et al.* (2005) recorded 24% germination in freshly collected seeds from India. On the other hand, Nasr *et al.* (2013) showed that about 50% of the freshly collected *D. viscosa* seeds from Iran germinated without any pre-treatment, although 45 min in sulphuric acid and 5 s in hot water improved germination to almost 90%. However, the result of Nasr *et al.* (2013) that 50% of the freshly collected *D. viscosa* seeds are non-dormant must be approached with caution. This is because, in their study, the moisture content of the seeds at the time of collection was not determined, thus there is a possibility that seeds used in their experiment might have been collected before full maturity.

Baskin *et al.* (2004) reported that seeds of *D. viscosa* stored dry under laboratory conditions for 1 year had failed to break dormancy. Also, Benítez-Rodríguez *et al.* (2013) stored seeds at room temperature for 2.5 months with no improvement in germination. Our results are in line with this. The duration required for breaking PY in seeds stored at room temperature not only varies between families but also within a family. Thus, while dry storage of several species with PY from the Fabaceae (Hu *et al.*, 2013), Geraniaceae (Meisert, 2002; Van Assche and Vandelook, 2006) and Malvaceae (Egley, 1976) lose dormancy during storage, some genera in the families including Geraniaceae (Van Assche and Vandelook, 2006) and Malvaceae (Egley and Paul, 1981; Van Assche and Vandelook, 2006) produce seeds that remain dormant even after 2 years of dry storage.

Furthermore, unlike seeds of *D. petiolaris*, which came out of dormancy at ecologically significant temperatures within 24 weeks of incubation, with the total percentage of seeds germinated increasing with increase in temperature: 20/35°C > 13/26°C >15°C > 10/20°C (also high temperature broke dormancy quickly) (Turner *et al.*, 2009), seeds of *D. viscosa* stored on a moist substrate at 10/20°C, 15/20°C, 15/30°C, 20/35°C and 25°C showed no improvement in germination even after 32 weeks (Fig. 2; see also Baskin *et al.*, 2004). A comparison of temperature and time requirements for rendering seeds of *Dodonaea* species non-dormant, reflects an extremely species-specific degree of hardseededness. For example, seeds of *D. aptera* and *D. hackettiana* displayed significant improvement in germination when wet heated for 1 h at 50°C and 60°C, respectively, but those of *D. ptarmicaefolia* and *D. viscosa* (this study) failed to do so at any temperature below 80°C (Cook *et al.*, 2008; Fig. 3). Similarly, seeds of *D. viscosa* subjected to dry heat for 60 min came out of dormancy at temperatures only above 80°C, although an increase in temperature decreased the required duration (Baskin *et al.*, 2004), but contrasting temperature requirements in other species of *Dodonaea* have been reported (Turner *et al.*, 2009).

According to Benítez-Rodríguez *et al.* (2013) immersing seeds of *D. viscosa* in sulphuric acid for 6–10 min can break dormancy in more than 90% of the seeds, but dipping seeds in hot water for 2.5–5 min was ineffective. However, the results of the present and earlier studies (Hodgkinson and Oxley, 1990; Baskin *et al.*, 2004; Phartyal *et al.*, 2005) are in sharp contrast to their conclusion, as hot water treatment for 30 s broke dormancy in almost 90% of the seeds tested (Fig. 2).

High temperature requirements to break dormancy in Sapindaceae, especially in the genus *Dodonaea*, have been tested in some of the Australian species (Cook *et al.*, 2008; Turner *et al.*, 2006, 2009). In particular, Cook *et al.* (2008) demonstrated that artificially buried seeds of *D. hackettiana* germinated to 36% when exhumed after 4 months, although seeds before burial were only able to germinate to 6%. In the present study, *D. viscosa* seeds buried in the soil for 6 months also germinated to 37 ± 5.1%, but 94% of the remaining non-germinated seeds were alive and germinated successfully after hot-water treatment. Seed germination of buried seeds improved progressively with increase in time (Fig. 4). However, all the seeds buried must have experienced more or less similar conditions since we buried them in the top soil layer. Nevertheless, this is a chance event in nature, because seeds do not germinate immediately after dispersal and persist for a long period. This observation provides some evidence to suggest that seeds of *D. viscosa* establish persistent seed banks because dormancy allows only a proportion of seeds to germinate every year, as observed in many Fabaceae species (Baskin and Baskin, 1998; Van Assche *et al.*, 2003; Van Assche and Vandelook, 2006; Hu *et al.*, 2009).

One of the most important findings of the present study was that seeds of *D. viscosa* displayed pronounced germination when exhumed after summer (in August) (Fig. 4). Additional evidence that dormancy is broken by summer temperatures also comes from a study conducted in Brazil, which showed that seeds of *D. viscosa* collected in January only germinated after scarification, but those collected in November were able to germinate to 93%, i.e. dormancy was broken in the field during summer and autumn (da Rosa and Ferreira, 2001, cited by Baskin *et al.*, 2004). In a 2.5-year soil seed bank study on 14 Fabaceae species, Van Assche *et al.* (2003) critically highlighted that most of the seeds germinated during spring after PY was broken by low temperatures in winter. In contrast to temperate locations, where many species require low winter temperature to break dormancy, low winter temperatures do not occur in

the wet–dry tropical environments (Fig. 4). Germination during June through September can have numerous advantages to *D. viscosa* seeds. Water requirements to complete initial stages of germination can be satisfied during the rainy season after summer. Furthermore, following the rainy season, moderate climatic conditions prevailing until summer provide ideal conditions for seedling growth.

Based on our data, we believe there may be three factors that would provide conditions favouring dormancy break in *D. viscosa* seeds in the soil: (1) dry heat – soil temperatures rising to 55–65°C during April to June; (2) moist heat – rainfall events moistening the seeds with subsequent temperatures rising above 50°C; and (3) daily fluctuating temperatures of 15–20°C in the months of May to July. However, which of these particular factors, or combinations, are required for seeds to lose PY remains to be shown. However, there is some indication that dormancy breaking occurs in wet soil at high temperature. Interestingly, Benítez-Rodríguez *et al.* (2013) found that *D. viscosa* seeds artificially buried for 2.5 months in a moist forest site, germinated to 22%, as compared to less than 5% at drier sites. In their study area, similar to our data, temperatures above 60°C are common. Hodgkinson and Oxley (1990) also found that soil temperatures rising above 70°C, as a result of fire, improved germination in *D. viscosa* seeds, mostly in moist soil. These studies suggest that the ability of seeds to break dormancy can be influenced to some extent by the micro-site. Perhaps the variation in micro-climate and soil environment between August and February can be a reason for the low germination seen in seeds buried in August.

While dry heat of 80°C for 1 h, and wet heat of 80°C for 5 s, can break dormancy in *D. viscosa* seeds, this is not even a chance event in the natural environment. However, although the dormancy-breaking effects of long-term exposure of seeds to temperatures between 40 and 60°C have not been studied in *D. viscosa* or in many PY species, we suggest that this is more likely an ecological route of dormancy breaking or pre-conditioning (see below). In alfalfa, seeds held at 41°C became permeable to water after 5 d (Ellis and Palmer, 1973). A moderate temperature of 55°C for 16 h was effective in breaking PY of *Mucuna aterrima* (Wutke *et al.*, 1995). Van Assche and Vandelook (2006) noted that summer temperatures may dry the seeds present in soil surface which alleviates PY and serve as a possible environmental cue for the winter annuals to germinate after summer, so that germination synchronizes with the most favourable condition.

Experiments with *Geranium carolinianum* (Gama-Arachchige *et al.*, 2012), *G. columbinum*, *G. dissectum*, *G. lucidum*, *G. molle* and *G. pusillum* (Van Assche and Vandelook, 2006), *Ipomoea lacunosa* (Jayasuriya *et al.*, 2008a), *Ornithopus compressus* L. (Taylor and Revell, 1999) and *Trifolium subterraneum* (Taylor, 1981) have shown that PY breaking involves a pre-conditioning step prior to the actual opening of the water gap. Identical dormancy breaking in Sapindaceae could therefore be expected, and there is some evidence to show that cooler conditions prior to the rise in summer temperatures act as a pre-conditioning step (Fig. 4). Importantly, pre-conditioning serves as an 'environmental detector' to sense the favourable time for opening the water gap. Thus, the seeds are conditioned at lower temperature and if favourable conditions (high temperatures) are not met, the pre-conditioning step is reversed and seeds continue to persist until next year (Baskin and Baskin, 2014). Future studies, for example similar to those conducted in Geraniaceae (Gama-Arachchige *et al.*, 2012), are imperative to predict the ecological significance of those factors determining the timing of germination in Sapindaceae seeds.

In general, mechanically scarified seeds germinated faster than other dormancy-breaking methods tested here and dry heat as indicated by Baskin *et al.* (2004). For example, only one hot-water treated seed germinated at the third day of incubation, but 19% of the mechanically scarified seeds germinated by this time (Fig. 1). We agree with Baskin *et al.* (2004) that water movement in mechanically scarified seeds occurs throughout the scarified regions, and that a slower imbibition rate in other treatments is explained by water movement only through specialized structures. Evidently, germination of seeds buried in the soil was also slow and some seeds started to germinate only during the second week, when most, if not all, mechanically scarified seeds completed germination. These results indicate that seeds break dormancy in the natural environment by opening specific gaps available on the seed coat for water entry. In a detailed study on *Dodonaea petiolaris* and *Distichostemon hispidulus*, Turner *et al.* (2009) identified the water gap in members of the Sapindaceae family to be a small plug in the seed coat, which is located adjacent to the hilum. There is a great likelihood that this plug may also act as the water gap in *D. viscosa.*

Germination of seeds during burial supports the paradigm that seeds of *D. viscosa* do not require light for radicle emergence (Burrows, 1995; Baskin *et al.*, 2004). However, Benítez-Rodríguez *et al.* (2013) reported that *D. viscosa* seeds buried at a depth of 5 cm in soil for 2.5 months failed to germinate until exhumed and germinated *ex-situ*. Consequently, they suggested that other soil factors, e.g. gases, prevented germination in soil. On the other hand, in the burial experiment conducted in Australia, all the seeds of *D. hackettiana* germinated during burial (Cook *et al.*, 2008). From the available evidence it is clear that most of the seeds with PY occurring in different environments can germinate at a wide range of temperatures under both darkness and

light, even from a soil depth of 7 cm, once the seed coat becomes permeable (Van Assche *et al.*, 2003; Van Assche and Vandelook, 2006; Hu *et al.*, 2009; Liu *et al.*, 2011). Thus, it seems likely that either the requirement of additional soil factors might have been satisfied in the study area or germination in soil occurs after 2.5 months.

In conclusion, we have shown that seeds of *D. viscosa* from the south of India are dormant. In addition, there is strong evidence to conclude that dormancy break occurs in the field due to high summer temperatures. This study, to our knowledge, is the first to evaluate the ecological dormancy loss in Sapindaceae over 2 years. In contrast to the spring germinators in temperate environments, which benefit by growing rapidly in summer, germination at the end of summer or early in autumn is highly adaptive to the tropical climate, so that seedlings grow actively to suitable size during the months of moderate temperature, thereby tolerating summer drought. The overriding role of season in controlling germination of other *Dodonaea* species and other members of Sapindaceae family having PY is currently under investigation.

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Conflict of interest

None.

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