Seed Science Research

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A wide range of lens morphologies is associated with breaking physical dormancy in *Paraserianthes lophantha* subsp. *lophantha*

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Research Paper

Cite this article: Burrows G (2025) A wide range of lens morphologies is associated with breaking physical dormancy in *Paraserianthes lophantha* subsp. *lophantha*. *Seed Science Research* **35**, 135–144. https://doi.org/10.1017/S0960258525100020

Received: 2 July 2025
Revised: 4 August 2025
Accepted: 5 August 2025
First published online: 19 August 2025

Keywords:
Acacia; germination; imbibition; testa; physical dormancy; water-gap; lens

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Abstract

The classification of acacias has gone through recent upheaval. The latest phylogenies indicate that *Acacia sensu stricto* is only relatively distantly related to the species with which it was once grouped. Its sister group is the monospecific *Paraserianthes*. This study concerns *P. lophantha* subsp. *lophantha*, a species from SW Western Australia that is widely invasive. Both genera have seeds with physical dormancy (PY) and a lens-type water gap. Seed structure, particularly that of the lens, was assessed in *Paraserianthes* and compared with *Acacia*. Seed batch viability was almost 100%, all seeds had PY and average seed mass was 73 mg. The seed coat and the embryo made almost equal contributions to seed mass, indicating a substantial seed coat. Average testa (410 µm) and palisade layer (163 µm) thicknesses were greater than in most investigated *Acacia* species. Unpopped lenses were small (0.11 mm^2, about 0.15% of the seed surface area). With a 1 min boiling water treatment, the lens detached from the seeds. The palisade cells of the lens were about 100% larger in area after detaching, which indicates that they previously were under considerable tension. With other PY-breaking treatments, the lens formed a mound or a slight change in colour occurred. The seeds of *Paraserianthes lophantha* had the same basic construction as most *Acacia* seeds, although they were relatively large and heavy, the testa made up a large proportion of the seed and the palisade cells were long. Different lens morphologies, associated with different dormancy-breaking treatments, have rarely been described.

Introduction

Legume classification has undergone significant changes in recent times. Once considered as three families, the legumes had become generally accepted as a single family (Leguminosae), with three subfamilies (Caesalpinioideae, Faboideae and Mimosoideae). In 2017, the family was split into six subfamilies (LPWG 2017), with the former subfamily Mimosoideae referred to informally as the mimosoid clade, within the re-circumscribed Caesalpinioideae (LPWG 2017; Bruneau et al., 2024). Within the legumes, the acacias and their close relatives (Mimoseae) are the focus of this study. The acacias are the second-largest genus of legumes. In the early 2000s, *Acacia sensu lato* had around 1400 species, but the acacias are now classified into seven genera (Murphy and Maslin, 2023). Recent phylogenetic studies show the six genera that were once included in *Acacia sensu lato* are now in different clades to *Acacia sensu stricto* (Bruneau et al., 2024). The sister taxon of *Acacia* is *Paraserianthes* (Brown et al. 2011; Bruneau et al., 2024).

Paraserianthes was composed of four species, but three of these have been placed in *Falcataria*, leaving *Paraserianthes* with a single species, *Paraserianthes lophantha* (Willd.) I.C.Nielsen (Bruneau et al., 2024). This taxon has been previously classified as a species in *Acacia, Mimosa* and *Albizia*. There are two subspecies: *P. lophantha* subsp. *lophantha* (native to SW Western Australia) and *P. lophantha* subsp. *montana* (native to Sumatra and Java and the Lesser Sunda islands of Bali and Flores) (Bruneau et al., 2024). The relationship between these geographically disjunct subspecies has not been tested, as the latter has not been genetically sampled (Bruneau et al., 2024). All subsequent references to *P. lophantha* in this study refer to *P. lophantha* subsp. *lophantha*.

Paraserianthes lophantha is a shrub to medium-sized mesophytic understorey tree from the temperate forests of SW Western Australia (Dell, 1980; Brown et al., 2020). It is an obligate reseeder, with its regeneration linked to fire (Brown et al., 2020). Its seeds can lie dormant in the soil for many years and germinate prolifically after fire (García-Duro et al., 2019). It is a significant weed, both in Australia (near coastal areas of South Australia, Victoria, NSW and Tasmania) and overseas (South Africa, Canary Islands, Chile, New Zealand, Portugal and southern California) (Brown et al., 2020).

The seeds of *P. lophantha*, like those of most species of *Acacia*, have physical dormancy (PY) (Table 1). In seeds with PY, the cells of the epidermal palisade layer (macrosclereids) of the

Table 1. Studies of the germination of *Paraserianthes lophantha*, arranged chronologically

Author	Comments
McDowell and Moll (1981)	Seeds from South Africa; seed weight 93 mg, MC 7.8%
Bell et al. (1993)	2% G in control; 40–51% G after 30, 60, 120 or 300 s BW
Bell et al. (1995)	Av. seed weight: 77 mg; viability 64%, G 80% after 60 s BW
Cromer (2007)	Same WA seed lot tested in 1990 and 2005; very low G in control, viability 70%; various BW treatments gave 40–74% G; 80°C dry heat for 45 min gave 9% G
Leino and Edqvist (2010)	Seed collected in Egypt in 1856. Assessed 151 years later. Seed mass 55 mg. 0% G from 33 seeds.
Channing (2011)	Most seeds used for experiments came from the soil seed bank, Auckland, NZ. Laboratory studies used trays filled with soil – dry and wet soils were treated at 40°C or 80°C (using heat lamps) for about 20 m. Dry 80°C and wet 80°C gave the best results (62% and 41% G, respectively). Relatively high G (25–40%) in controls.
Harris et al. (2017)	Compared seven populations in native range (SW WA) and nine populations in coastal southern Australia (CSA). Used a 1 m BW treatment to reduce PY. Seed predation was very high in WA and very low in CSA. Seed viability was the opposite. Time to first germinant was quick in both populations and much faster than the four acacias also examined. Dispersal investment (elaiosome) was minimal in both populations. Average seed weight was about 27 mg in WA and about 90 mg in CSA.
García-Duro et al. (2019)	Seeds from Spain with an average maximum length of 4.5 mm. Among various treatments (e.g. ash, charcoal and smoke), dry heat (80°C, 110°C, 150°C and 200°C for 5 and 10 min) was also used. The best treatments were 80°C for 5 and 10 min and 110°C for 5 min, but these only gave 10–16% G. At 150°C and 200°C, seeds were killed.

BW, boiling water; G, germination; MC, moisture content.

testa are impenetrable by water. Thus, when surrounded by a moist substrate, the seeds do not imbibe (Burrows et al., 2018, 2019). In nature, the heat of a fire can break PY in legumes, while in nurseries treatments such as hot water (HW) and boiling water (BW), along with mechanical and chemical scarification, are often used to break PY. While it is often mentioned that HW and BW treatments 'soften' the seed coat (also referred to as the testa), they only affect a small specialized part of the seed coat – the water-gap. A range of water-gap complexes has been described, with acacias being classified as having a Type II (simple) lens gap (Gama-Arachchige et al., 2013; Geneve et al., 2018). In acacias, the palisade cells of the lens are (i) shorter than in the rest of the testa, (ii) bowed outwards and (iii) attached to one or two layers of thin-walled, easily broken, parenchyma cells (Dell, 1980; Hanna, 1984; Burrows et al., 2009). With the thermal shock of hot air or hot water, the palisade cells of the lens detach from the weak underlying cells and rapidly expand outwards, creating a gap through which water can subsequently enter the seed. In acacias, externally the lens is usually less than 300 µm in diameter and, after popping, the gap in the testa is even smaller (Burrows et al., 2018). The unpopped lens in *Acacia* seeds is usually only about 0.1% of the surface area of the seed and can be easily overlooked (Burrows et al., 2018).

Burrows et al. (2018, 2019) examined testa and lens structure in about 50 species of *Acacia*. While this is only about 5% of all Australian *Acacia* species, it is by far the most extensive study of the lens, before and after a PY-breaking treatment. Vassal (1971) studied seeds of about 130 *Acacia sensu lato* species, both Australian and African. He measured seed dimensions, with ranges and averages given for all species. He also measured lens ('trace rapheale') length and testa thickness, but data were not given for each species. This information was presented as ratios (hilum length/lens length and seed length/testa thickness), that were arranged into groups.

Few studies of seed structure in *P. lophantha* have been made (Dell, 1980; Gunn, 1984; Lersten et al., 1992). Several studies have been conducted on the relative effectiveness of PY-breaking treatments (Table 1), although none of these studies relate the effectiveness of the treatments to lens structure. Burrows et al. (2009, 2018, 2019) have shown that a quick examination of the lens under a dissecting microscope, both before and after various PY-breaking treatments, can be useful when interpreting imbibition and germination results.

Given its invasive nature, I considered it worthwhile to examine the germination of *P. lophantha* seeds, after a range of PY-breaking treatments, especially in terms of lens structure. As *Paraserianthes* is the sister group of *Acacia*, it was also interesting to assess the similarities and differences of the two genera in terms of seed structure and biology.

Materials and methods

Seeds of *P. lophantha* were obtained from a commercial supplier. The seeds had been collected near Albany, SW Western Australia in 2024. A small percentage of seeds was much thinner than the other seeds and these were not used in the experiments. The seed batch was almost entirely free from insect attack.

Structure

1. Morphology: seed surfaces and associated features (e.g. elaiosome (if present), funiculus, hilum, micropyle and pleurogram) were examined with a Nikon SMZ25 dissecting microscope.

2. Seed size, volume and surface area: length and width of control and fully imbibed, but not germinated, seeds were measured externally with a Nikon SMZ25 dissecting microscope, using NIS Elements software. Thickness (depth) was measured after fracturing the seeds open halfway between the proximal and distal ends, in a transverse plane. Embryo size was also measured. Twenty-two seeds were measured.

Volumes of the whole seed, testa and embryo, in control and imbibed seeds, were estimated using an online application to determine the volume of an ellipsoid. For these estimations, it was assumed that the testa had an even 410 µm thickness in control seeds and 505 µm in imbibed seeds (Table 2). These dimension-based estimates were checked with water displacement measurements. Three replicates of 10 control seeds were placed in a 10 ml graduated cylinder with a known volume of water. The increase in volume was measured and converted to an average volume per seed in mm^3. After a HW PY-breaking treatment, the seeds

https://doi.org/10.1017/S0960258525100020 Published online by Cambridge University Press

Table 2. Various measurements of *Paraserianthes lophantha* seeds from Dell (1980) and the current study

Parameter	Dell (1980)	Current study
Seed dimensions – C (mm)	2.5 × 3.0	6.9 ± 0.3 (L) × 4.6 ± 0.2 (W) × 3.1 ± 0.2 (D)
Seed dimensions – I (mm)	5.3 × 6.3	10.6 ± 0.4 (L) × 6.1 ± 0.3 (W) × 4.8 ± 0.3 (D)
Est. volume of seed (mm^3)		51 (C), 166 (I)
Est. surface area of seed (mm^2) – C		73
Volume of C testa/embryo (mm^3)		23.8 (47%)/ 26.8 (53%)
Volume of I testa/embryo (mm^3)		66.5 (40%)/99.5 (60%)
Seed mass – C and I (mg)	c. 65 (C)	71.0 ± 6.2 (C), 182 ± 15 (I)
Testa, embryo mass – C (mg)	c. Testa 35, embryo 30	Testa 36 ± 2 (51%) Embryo 35 ± 3 (49%)
Testa, embryo mass – I (mg)	Testa 75–80 (121%), embryo 90–95 (208%)	Testa 84 ± 7 (133% increase), embryo 98 ± 9 (180% increase)
Testa thickness (μm)	c. 410 (C); 646 (I)	410 ± 25 (C), 511 ± 18 (I)
Cuticle thickness (μm)	11.6 ± 0.6	16 ± 3 (C), 12 ± 3 (I)
Palisade thickness (μm)	132.9 ± 0.9	163 ± 18 (C), 180 ± 7 (I)
Mesophyll thickness (μm)		231 ± 19 (C), 319 ± 17 (I)
Palisade thickness (μm) in lens	70–75	69 ± 7
Lens dimension (μm) – external (C)	520 × 300	370 ± 30 (L) × 385 ± 35 (W)
Lens dimension (μm) – in testa – after BW	250–300 × 150	191 ± 21 (L) × 189 ± 32 (W)
MC seeds (C)		9.6%
MC of testa and embryo	Reduces to 8.7% (T)	11.5% (T), 6.9% (E)

C: control, I: imbibed. Average ± SD, MC – moisture content on fresh weight basis; L, length; W, width; D, depth; BW, boiling water; T, testa; E, embryo; est., estimated; inc, increase.

were imbibed and remeasured. The imbibed seeds were then separated into testa and embryo components and these were measured. The surface area of control seeds was estimated using an online application for the surface area of an ellipsoid.

3. Seed mass: individual seeds were weighed on a balance accurate to four decimal places (80 seeds). The percentage of seed mass composed of the testa and the embryo was also calculated (12 seeds). This was repeated for fully imbibed, but not germinated, seeds.

4. Seed moisture content (MC) on a fresh weight basis: 10 whole seeds and 10 seeds fractured into halves for quicker moisture loss (Burrows, 2024) were weighed, then placed in an oven at 103°C and weighed after various intervals. Final MC was calculated after 24 h, which is a slight modification of the International Seed Testing Association's low constant-temperature drying method (103°C for 17 h). In addition, 10 seeds were divided into their testa and embryo components and MC was determined as described earlier.

5. Testa structure: seeds were fractured in transverse section (TS) with a no. 22 scalpel blade. The seeds could not be safely cut by hand pressure alone, so the blade was gently tapped with a hammer. The thickness of the whole testa, cuticle, palisade (macrosclereids) and mesophyll plus hourglass cells was measured in two places in each of 30 seeds using a Nikon SMZ25 microscope. This was repeated for imbibed, but not germinated, seeds. The testa was also hand-sectioned in TS, with the sections mounted unstained in water on glass slides. These sections were then examined using bright field microscopy and a Nikon Eclipse Ni microscope.

6. Lens structure: external lens length (in the same line as the micropyle and hilum) and width were measured in untreated seeds. Seeds were also given a BW treatment (100°C for 1 min). In these seeds, the lens popped off as a plug that usually remained loosely attached to the seed (Figure 2e). The following parameters were then measured: (i) exterior (essentially the cuticle) and interior dimensions (inner side of the palisade cells) of the popped lens and (ii) dimensions of the gap in the testa caused by the lens popping. Thirteen seeds were assessed. The area of the gap in the testa and the inside of the popped lens were calculated (area of an oval) and also measured in situ using Nikon Elements software.

Seed imbibition

Rates of imbibition of control seeds, 1 min BW-treated seeds and seeds that had been nicked near the distal end were measured. The nick extended into the mesophyll cells, but not into the cotyledons. The individual seeds were weighed, then placed on a moistened paper towel in 9 cm diameter Petri dishes, at 22°C, and weighed at various intervals until they were fully imbibed. Ten seeds were used for each of the three treatments.

Paraserianthes lophantha has relatively large seeds compared to many *Acacia* species. It appeared that in seeds placed on top of moist filter paper (as is standard in many germination studies), the lens would be several millimetres away from the contact point with the moist substrate. In addition, between the lens and the point of contact was the potentially water-repellent cuticle. Thus, in seeds with a popped lens, moisture might not be efficiently transported to the lens; thus, imbibition might not occur (or would occur slowly). To investigate this possibility, seeds were given a 1 min BW treatment. After the seeds had dried they were individually weighed. The seeds were then checked under a dissecting microscope to confirm that the lenses had completely detached. Four replicates of five seeds were then (a) placed on top of eight thicknesses of moistened paper towel, (b) placed on seven thicknesses of moistened paper towel, with one layer placed over the

https://doi.org/10.1017/S0960258525100020 Published online by Cambridge University Press

top of them, or (c) placed in shallow water in a Petri dish so that they were completely submerged. The seeds were then weighed at 24 h intervals until fully imbibed (around 160% increase in mass). While the full results are presented below, the 7 + 1 layers technique gave relatively rapid imbibition, along with the convenience of seeds not moving about in a liquid medium. This method was used in all subsequent imbibition and germination experiments.

PY-breaking treatments and seed germination

Moist heat

Water temperature 1: Three replicates of 10 or 11 seeds were placed in a stainless-steel tea infuser, then plunged into water at 40°C, 50°C, 60°C, 70°C, 80°C or 90°C for 1 min, along with a control treatment. After treatment, seeds were placed in 9 cm Petri dishes with a moistened paper towel as described earlier, then placed in an incubator at 22°C (in the dark). After 24 h the lens of each seed was inspected under a dissecting microscope to determine if the lens was apparently unchanged, had formed a mound or had completely detached as a disk. The seeds were then assessed almost daily to record if they had imbibed (easily visually assessed as imbibed seeds were much larger and were brown rather than black) and/or germinated.

Water temperature 2: Three replicates of 10 or 11 seeds were placed in a stainless-steel tea infuser, then plunged into BW for 1–2, 10 s, 1, 5, 10 or 20 min, then processed as described earlier. After 24 h, the lens of each seed was inspected under a dissecting microscope to determine if the morphology of the lens had changed (as described earlier). The seeds were then assessed almost daily to record if they had imbibed and/or germinated. The occurrence of bacterially infected seeds was also monitored. The presence of bacterial infection could be found within 72 h and was often noted as a ring of milky-coloured liquid along the pleurogram.

Steam: Three replicates of 10 seeds in a stainless-steel tea infuser were shaken while placed in the steam coming from the spout of a boiling kettle for 15 s. The morphology of the lenses was checked; then the seeds were processed as described earlier.

Dry heat

Oven at 100°C: Three replicates of 10 seeds were placed in aluminium foil trays, then placed in an oven at 100°C for 0, 2, 5, 10, 30 or 60 min. The morphology of the lenses was checked, then the seeds were processed as described earlier.

Bunsen burner: Three replicates of 10 seeds were used, with individual seeds held by forceps at the edge of a Bunsen burner flame, hilum end in, for 1–2 s then allowed to cool. The lenses were then assessed using a dissecting microscope to determine morphology. The seeds were then processed as described earlier and assessed on subsequent days to determine if the lens morphology had changed and if the seeds had imbibed and/or germinated.

Alcohol flame: 2 ml of 70% ethanol was sprayed on a sheet of stainless steel and then ignited. Three replicates of 10 seeds in a stainless-steel tea infuser were then shaken near the tip of the flames for 5 s (a modification of the 'Flaming Fabaceae' technique, described by Sugii, 2003). The seeds were then processed as per the Bunsen burner experiment. Seeds that were still unimbibed after 20 days were nicked, placed on a moist paper towel and assessed after 4 days to check their viability.

Liquid nitrogen (LN): Three replicates of 10 seeds were immersed in LN for 5 or 20 min. They were then removed and allowed to gradually return to room temperature. They were then processed as described earlier. Seeds that were still unimbibed after 20 days were nicked, placed on a moist paper towel and assessed after 4 days to check their viability.

Combination: The various dormancy-breaking treatments produced different modified lens morphologies and germination percentages (see below). To investigate their influence on imbibition and germination in a single experiment, the following treatments were applied to three replicates of 10 seeds: (i) control, (ii) BW 1 min, (iii) nicked with scalpel, (iv) Bunsen burner flame 1–2 s and (v) EtOH flame 5 s. Lens morphology was examined after treatment, then the seeds were processed as described earlier.

Data and statistical analysis

Four data sets were collected, based on four different study designs, to quantify the percentage of seed imbibition and germination after specified time periods (see Tables 3–6 for further details). In the first two experiments, the percentage of germinated seeds was assessed on the 7th and 14th days. In the third experiment, the percentage of imbibed seeds was assessed after 7 days and percent germination after 14 days. In the final experiment percent germination was assessed after 4 days. Since the response variables were proportions of the germinated seeds, binary logistic regression models (one of the most popular generalized linear regression models) were employed for inferential analysis (Crawley, 2013). In the data sets, we observed 0% or 100% germination or imbibition with some data points, which leads to undefined values when using a logit link function due to infinite log-odds. To address this, while preserving the continuous nature of germination probability as a gradual biological response, we applied a small adjustment, replacing proportions of 0 and 1 with 0.01 and 0.99, respectively, before the model fitting step. This method is a practical approximation when modelling gradual biological responses and has a precedent in ecological and statistical literature (Agresti and Coull, 1998; Warton and Hui, 2011). It was decided that seven binary logistic regression models (each with the treatment variable as the only predictor) were fitted to the data that were collected on the same day. The model fitting was conducted using the R statistical software (R Core Team, 2024). The post-hoc analysis for obtaining the pairwise comparison hypothesis testing results was performed using the R function emmeans (estimated marginal means) from the special R package emmeans (Lenth, 2024).

Results

Through the results of several experiments, the control seeds were shown to be almost 100% viable, with 100% PY, and they remained unimbibed, but 100% viable, after several weeks on a moist substrate.

Seed structure

1. Seed morphology: seeds were elliptical, and the testa was black but with a whitish bloom from small flecks of wax (Figure 1a). A pleurogram that was open at the hilum end of the seed was present on both flattened sides of the seed (Figure 1a). An elaiosome (aril) was not present. Most of the funiculus had detached during seed processing, but a small stub usually remained attached to the seed (Figure 1a), and thus, a hilum was usually not observed. The persistent base of the funiculus curved back over the micropyle, i.e. away from the lens (Figure 2b–f). A ring of smooth tissue or

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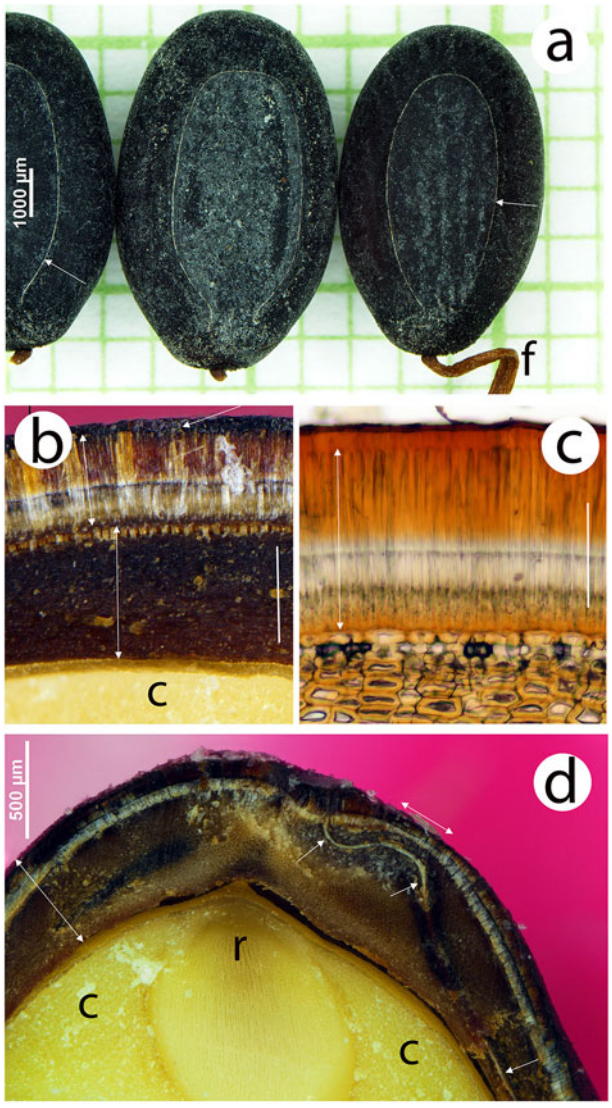

Figure 1. External morphology and images of the internal structure of the seed coat of *Paraserianthes lophantha* subsp. *lophantha*. (a) Seed morphology showing the pleurogram (arrows) and funiculus (f) joined to the seed at the hilum. Background grid is in mm. (b) Transverse section (TS) of the seed coat away from the hilum and lens region. Single-headed arrow – cuticle; shorter double-headed arrow – palisade layer; longer double-headed arrow – mesophyll layer with outer layer of hour-glass cells. c – cotyledon. Scale bar 200 µm. (c) Unstained hand section of a TS of the testa. The double-headed arrow indicates the palisade layer. The cuticle is on the outside of this, the hourglass cells immediately to the inside. Note the deep orange staining in the cuticle and outer half of the palisade layer. Note also the unstained zone to the inside of the light line. Scale bar 100 µm. (d) Longitudinal (sagittal) section in the plane of the micropyle, hilum and lens. Note the vascular bundle (single-headed arrows) that enters at the hilum, curves inwards at first then curves outward (just under the lens – short double-headed arrow), before going deep within the testa (long double-headed arrow). c – cotyledon, r – radicle.

cuticle, about 700 µm in diameter (the ring or band was about 270 µm wide), surrounded the funiculus stub (Figure 2b–f).

2. Seed size, volume and surface area: Control seeds were, on average, 6.9 × 4.6 × 3.1 mm in length, width and thickness, respectively (Table 2). Fully imbibed seeds were, on average, 10.6 × 6.1 × 4.8 mm in length, width and thickness, respectively (which represented 54%, 33% and 55% increases when imbibed, respectively) (Figure 2a, Table 2). Control and fully imbibed seeds had average approximate volumes of 51 and 166 mm^3, respectively (an approximate 225% increase when imbibed). Assuming the testa had an even thickness of 410 µm (see below), then the control testa and embryo had average volumes of 24 (47% of seed volume) and 27 mm^3 (53%), respectively. Assuming the testa of imbibed seeds had an even thickness of 504 µm (see below), then the imbibed testa and embryo had average volumes of 67 (40%) and 100 mm^3 (60%), respectively. These approximate volumes and proportions, based on dimensions, were confirmed by the water displacement measurements. The average control seed surface area was 73 mm^2.

3. Seed mass: average control fresh seed mass was 71.0 mg. The range, based on 80 seeds, was 59-86 mg. On average, the testa was 36 mg (51% of the mass) and the embryo was 35 mg (49%). In fully imbibed, but not germinated, seeds, the seed mass increased to, on average, 182 mg (a 156% increase). On average, the testa and embryo increased to 84 mg (a 133% increase) and 98 mg (a 180% increase), respectively.

4. Seed MC: the average control seed MC was 9.6%. The testa, on average, had an MC of 11.5% and the embryo 6.9%.

5. Testa structure: in control seeds, the average thickness of the testa was 410 µm (Figure 1b,c). The average thicknesses of the cuticle, palisade and mesophyll (plus hourglass cells) were 16, 163 and 231 µm, respectively (Table 2). The palisade layer had a distinct light line (Figure 1b,c). Freehand, unstained sections of the testa (Figure 1c) showed that most of the testa was a deep orange/brown, except for an almost completely unstained zone below the light line in the palisade mesophyll (Figure 1c). Longitudinal sections of the seed showed that the vascular bundle that entered the seed at the hilum curved about halfway into the testa, then curved outwards to arc just under the lens, before progressing inwards again, close to the embryo (Figure 1d). In seeds that had been BW treated the cream-coloured vascular bundle could be seen in the gap in the testa where the lens had been.

In fully imbibed, but not germinated, seeds testa thickness averaged 511 µm, with average cuticle, palisade and mesophyll thicknesses of 12, 180 and 319 µm, respectively, i.e. increases of −25%, 10% and 38%, respectively.

6. Lens structure: on average, in control seeds viewed externally, the lens was roughly circular (370 µm [length] by 385 µm [width]) (Figure 2b). In the seed batch used, the lens was not that distinct (Figure 2b), but in some other batches examined, it was much clearer. When popped from various dormancy-breaking treatments, the lens took various forms (Figure 2c–f). The response to an EtOH flame was variable. In a small percentage of seeds, the lens did not change or formed a mound. In most seeds, the lens became a slightly lighter shade of brown and had a glossier appearance (Figure 2c). With the heat from a Bunsen burner flame the lens formed a small golden mound (Figure 2d). After BW treatment it almost completely detached as a disk, often remaining attached by only a few cells (Figure 2e). The cuticle of the popped lens had a scalloped edge (Figure 2e,f) and a greater diameter than the inner part of the lens (Figure 2e,f). The palisade cells in the lens (control seeds) were, on average, 69 µm in length, 58% shorter than in the rest of the seed coat (Table 2). After popping the outer diameter of the lens was little changed. After popping, the palisade cells of the lens (about 280 µm diameter, about 0.062 mm^2 area) had an area about twice (121%) that of the gap (about 190 µm diameter, about 0.028 mm^2 area) created in the testa (Figure 2f).

Seed imbibition

The control seeds increased in mass by about 2% in the first hour, with no further increase during the remainder of the experiment. The nicked seeds were fully imbibed (about 160% increase in mass)

https://doi.org/10.1017/S0960258525100020 Published online by Cambridge University Press

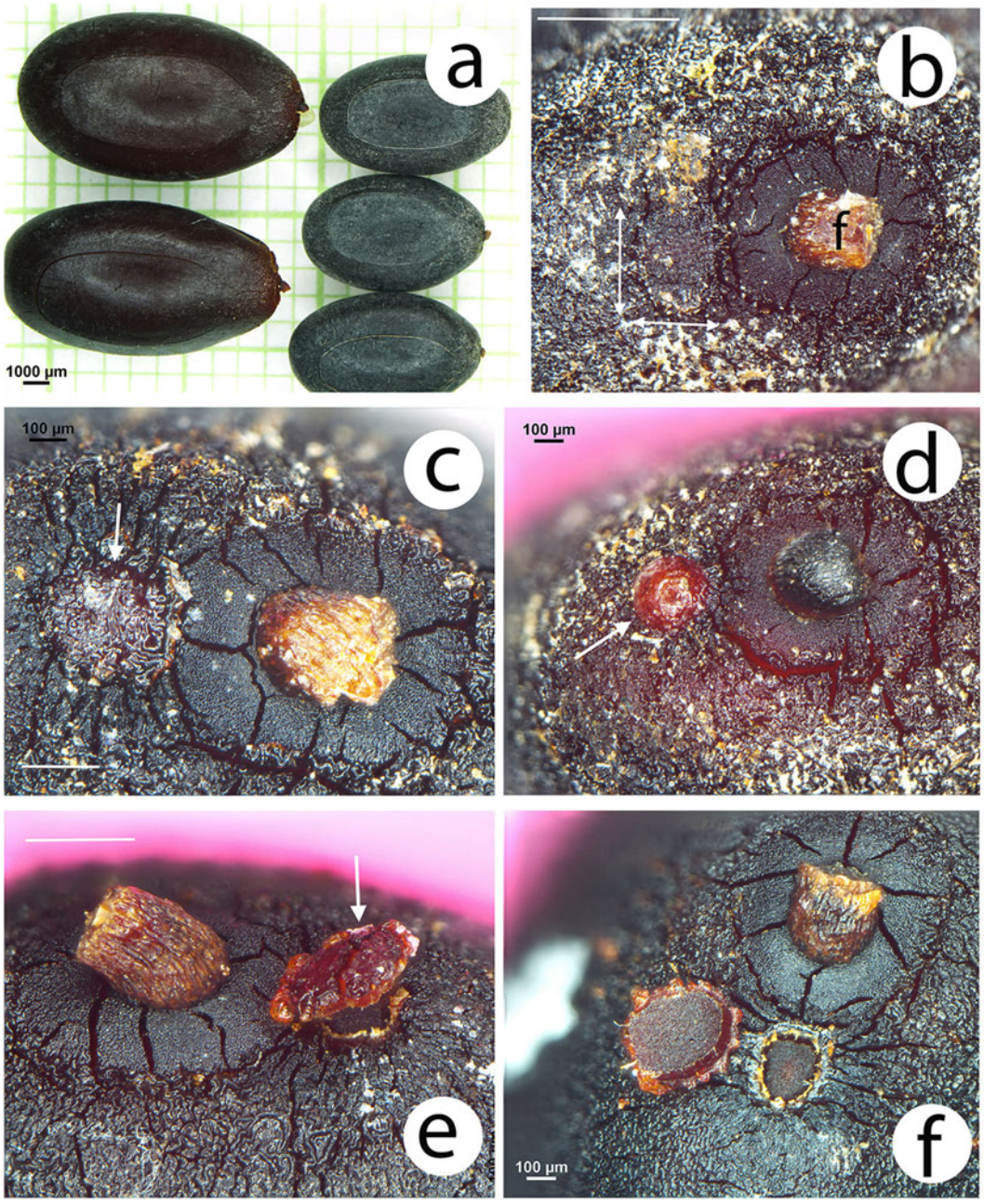

Figure 2. Seeds of *Paraserianthes lophantha* subsp. *lophantha*. (a) Morphology of fully imbibed seeds (left-hand side) and control seeds (right-hand side). Background grid is in mm. (b–f) Lens morphology. (b) Control seed showing the approximately circular outline of the lens (arrowed) and that the lens is relatively similar in external appearance to the rest of the testa's exterior. f, stub of funiculus. Scale bar 500 µm. (c) Lens after 5 s in an EtOH flame. Note the lens (arrowed) is slightly lighter in colour and glossier, but has not popped. White scale bar 200 µm. (d) Lens (arrowed) after 1 s in a Bunsen burner flame. The lens has popped, forming a golden-coloured dome. Scale bar 100 µm. (e) Lens after 1 min of boiling water treatment. Note the lens (arrowed) has completely detached as a disk. Scale bar 300 µm. (f) As per e, except the lens has been turned over. Note the scalloped edges of the cuticle and that the area of the palisade of the lens is now about twice the area of the gap in the testa.

after 48 h and were more than 90% germinated after 72 h. The BW-treated seeds reached these milestones about 24 h later. The average surface area of control seeds was about 73 mm^2. The average area of the gap in the palisade cells from a popped lens (Figure 2f) (popped as a disk) was 0.028 mm^2 (0.04% of the seed surface area), while the average area of mesophyll cells exposed by a scalpel blade nick was 0.5 mm^2 (0.7% of the seed surface area) (i.e. on average, 20× larger than the gap created by a disk popped lens).

Seeds (with popped lenses) placed on top of the paper towel, under one layer of paper towel, and submerged in water were fully imbibed, on average, after approximately 100, 70 and 50 h, respectively. The average germination, in all treatments, was at least 85% after 72 h. The average length of emerged radicle for seeds on top of the paper towel, under one layer of paper towel, and submerged in water after 72 h was 1.6, 6.4 and 1.9 mm, respectively.

https://doi.org/10.1017/S0960258525100020 Published online by Cambridge University Press

Table 3. Response of *Paraserianthes lophantha* seeds to 1 min exposure to water at different temperatures

Water temperature (°C)	Lens morphology after 1 day	G at 7 days	G at 14 days
Control	NC 100%, M 0%, D 0%	0^{c}	0^{c}
40	NC 100%, M 0%, D 0%	0^{c}	0^{c}
50	NC 58%, M 42%, D 0%	30^{b}	45^{b}
60	NC 6%, M 85%, D 9%	55^{b}	97^{a}
70	NC 0%, M 44%, D 56%	94^{a}	100^{a}
80	NC 0%, M 18%, D 82%	100^{a}	100^{a}
90	NC 0%, M 0%, D 100%	100^{a}	100^{a}

Lens morphology – NC: no change; M: mound; D: disk; G: average germination percentage; Different superscript letters in the same column indicate a significant difference between treatments at $p < 0.05$.

Table 4. Response of *Paraserianthes lophantha* seeds with exposure to boiling water (BW) for different times

Time in BW	Lens morphology after 24 h	G at 7 days	G at 14 days
1–2 s	M 74%, D 6%	70^{b}	97^{a}
10 s	M 30%, D 70%	100^{a}	100^{a}
1 min	M 0%, D 100%	100^{a}	100^{a}
5 min	M 0%, D 100%	72^{b}	$75^{a,b}$
10 min	M 0%, D 100%	52^{b}	52^{b}
20 min	M 0%, D 100%	46^{b}	45^{b}

Lens morphology – M: mound; D: disk. G: average germination percentage; Different superscript letters in the same column indicate a significant difference between treatments at $p < 0.05$.

PY break/seed germination

Moist heat

Water temperature 1: The lens was intact in all control seeds at 0 and 14 days. All control seeds spent 14 days surrounded by a moist paper towel without any imbibition. Increasing the temperature of the 1 min HW treatment was correlated with increasing modification of the lens (Table 3). All seeds with a modified lens had imbibed and germinated by day 14. At day 4, 99% of seeds with a disk-type lens had germinated, while only 71% of the mound-type lens seeds had germinated. Two seeds with no discernible change in lens morphology germinated, both after the 50°C treatment.

An interesting observation was that at the hotter water temperatures the seeds made a distinct 'click' as soon as they were immersed in the water. The sound was surprisingly loud for such a small object and could be clearly heard several metres away. The 'click' was almost certainly the sound made when a lens popped, especially when a detached disk was formed.

Water temperature 2: Even the shortest exposure to BW resulted in the lenses popping (Table 4). The 1–2 s exposure resulted in mostly 'mounds', 10 s mostly 'disks' and 1, 5, 10 and 20 min all 'disks' (Table 4). All the 10 s–20 min exposure seeds had imbibed after 48 h. Of the 1–2 s treated seeds 30% still had not imbibed after 7 days, but almost all had imbibed and germinated after 14 days (Table 4). All 10 and 60 s treated seeds had germinated after 4 days. The 5, 10 and 20 min BW treatments resulted in 75%, 52% and 46% germination, respectively. All non-germinated seeds were bacterially infected and never germinated. While about 50% of the 10 and 20 min in BW seeds met the condition for having germinated (radicle extension of > 2 mm), the seedlings were much less vigorous compared to the 10 and 60 s BW treatments.

Steam: The lenses of all seeds popped and formed a detached disk after 15 s in steam. All seeds had imbibed after 1 day and all seeds had germinated after 3 days.

Table 5. Response of *Paraserianthes lophantha* seeds to exposure to dry heat (100°C) for different times

Time (min) at 100°C	Lens morphology after treatment	Lens morphology at 24 h	I at 7 days	G at 14 days
0	NC	NC	0^{b}	0^{a}
2	NC	NC	0^{b}	0^{a}
5	NC	NC	0^{b}	0^{a}
10	NC	NC	7^{b}	3^{a}
30	NC	60% D	57^{a}	13^{a}
60	27% M	97% D	90^{a}	10^{a}

I: average percent imbibed, G: average germination percentage; Lens morphology: NC, no change; M, mound; D, disk. Different superscript letters in the same column indicate a significant difference between treatments at $p < 0.05$.

Dry heat

Oven at 100°C: Based on observations of the seeds immediately after the oven treatment, it appeared that even extended periods at 100°C had not affected the lens. However, when observed 24 h later, after the seeds had been placed on a moist paper towel, the lens structure had been altered in most seeds in the 30 and 60 min treatments (Table 5). In almost all seeds that had popped, the lens was in the form of a detached disk. Almost all the imbibed seeds had severe bacterial infection after 7 days, and none of these infected seeds germinated.

Bunsen burner: the lenses of all seeds popped, forming a small mound (Figure 2d). After 3 days, 82% of seeds had imbibed, and after 6 days 88% had germinated. Most seeds made a small 'click' when they entered the flame.

EtOH flame: this treatment did not result in the formation of mounds or disks but in about 50% of seeds the lens appeared a slightly lighter colour and was glossier (Figure 2c). After 6 days, 55% of seeds had imbibed and 48% germinationed. Scarification of the unimbibed seeds resulted in 100% germination.

Liquid nitrogen: for both the 5 and 20 min immersions no change to lens morphology was observed. After 12 days, no imbibition had occurred. Seed scarification resulted in 100% germination 4 days later.

Combination: The change to lens morphology and germination percentages was very similar to the previous application of these treatments (Table 6). Nicking, BW and Bunsen burner flame all reached over 90% germination after 4 days (Table 6). They reached their maximum germination percentages after 3, 4 and 7 days, respectively.

Discussion

Perhaps the most interesting aspect of this study was the variability in popped lens morphology, both between the different types of treatments (e.g. BW, Bunsen burner flame, EtOH flame and steam; Figure 2) and different temperatures and durations of the same type of treatment (i.e. HW and BW). Variation ranged from:

(i) initially no visible change was obvious but the lens popped some time later,

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Table 6. Response of *Paraserianthes lophantha* seeds to various treatments to break physical dormancy

Treatment	Lens morphology after treatment	G at 4 days
Control	Unchanged	0[c]
1 min BW	100% detached disk	100[a]
5 s EtOH flame	100% changed colour, 0% popped	47[b]
1 s Bunsen flame	100% small mound	93[a]
Nicked	Unchanged	100[a]

G: average germination percentage. Different superscript letters in the same column indicate a significant difference between treatments at $p < 0.05$.

(ii) the lens did not pop and showed only a slight change of colour and glossiness (e.g. after 5 s in an EtOH flame),
(iii) the lens formed a dome (e.g. after a Bunsen burner flame) to
(iv) the lens made a distinct click as it detached completely from the seed (e.g. after 1 min BW).

A similar report has been made for the seeds of *Albizia julibrissin* (Mimosoid clade) by Yang et al. (2020). They reported that with wet heat the lens opened 'in a circular, lid-like opening' (p. 582) (a 'detached disk' in this study), while dry heat 'pulled the lens cells apart' (p. 578) creating a 'non-circular, lid-like opening' (p. 578), also described as a 'semi-circular, lid-like opening' (p. 582). This latter form of lens popping looked similar to what I termed 'a mound'. The popped lenses in figure 4e,f of Yang et al. (2020) (oven heat) and Figure 2d (Bunsen burner flame) in the present study are quite similar. Yang et al. (2020) indicated that this was the first time this type of opening shape had been documented in the Mimosoideae. Burrows et al. (2009) illustrated that moist heat could modify the lens of *Acacia melanoxylon* into an intact mound, a mound with a crack exposing the palisade cells, a cylindrical tube and a detached disk (Burrows et al., 2009, Figure 1). In a similar manner, Burrows et al. (2018, fig. 4), across 51 *Acacia* species, described a range of lens morphologies in response to a single moist heat treatment. This leads to the question: why does BW (100°C) result in complete lens detachment, while the almost certainly higher temperature of the Bunsen burner flame only resulted in a small mound?

In the 51 *Acacia* species examined by Burrows et al. (2018), complete detachment of the lens after a BW treatment was only recorded in a few species and, even then, did not occur in all seeds in a seed batch. Other studies have recorded complete lens detachment in the Fabaceae (Lersten et al., 1992). In the discussion of Dell (1980), it was mentioned that the strophiolar plug (lens) swells to nearly three times its original surface area after being ejected following a hot water treatment. In the present study, the outer lens diameter was about the same (370 µm) before and after popping from a BW treatment. The resulting hole in the testa from lens detachment was only about 190 µm in diameter (0.028 mm^2 in area), while the palisade cells that once were in that space expanded to about 280 µm in diameter (0.062 mm^2 in area). This was about a 120% increase in area, indicating the cells were under considerable compression in the control seeds. Images of popped lenses, especially where a tube is formed (Burrows et al., 2009, 2018), also indicate that substantial lateral expansion of the palisade cells occurs after a lens has popped.

Paraserianthes lophantha has a relatively big seed compared to most acacias (surface area about 73 mm^2) and the lens opening, after a detached disk, is very small (0.028 mm^2) (about 0.04% of the seed surface). Nonetheless, at least under laboratory conditions, imbibition and germination occurred in a relatively short period. The size difference between control and imbibed seeds (Figure 2a) indicates that once imbibition starts the increase in embryo volume could generate cracks in the testa, leading to imbibition occurring in places other than the lens.

A wide range of treatments has been used to break PY in legumes (e.g. HW, BW, steam, LN, impaction, H_2SO_4, dry heat, scarification from sandpaper and scalpels). HW and BW are probably the most commonly used nursery methods to break PY, certainly for species in the Mimoseae. The mode of action of HW and BW needs to be understood. Papers are still published where these treatments are indicated 'to soften the seed coat', rather than specifically mentioning altering the lens. As long as short durations (< 5 min, while in most cases only a few seconds exposure to BW is all that is needed) are used, they result in rapid imbibition (although see Burrows et al. (2019) on delayed imbibition in *Acacia*) and high germination percentages. Longer exposures can result in damage to the embryo. While HW and BW treatments are safe and convenient for nursery propagation, they are also often used as a PY-breaking treatment in ecological studies. However, on the basis of this study, it appears that BW treatment may result in a popped lens morphology that may not occur in nature. Treatments with LN and dry heat in an oven (100°C) were unsuccessful. Other treatments used in this study, such as LN, can be effective for breaking PY (Salomao, 2002; Fernández et al., 2021). In this study, it did not pop the lens, but the seeds remained 100% viable. Cavanagh (1987a) notes that dry heat is generally less effective than HW/BW for legume seeds, although his appendix 4B lists several *Acacia* studies where it was effective (Cavanagh, 1987b). In *P. lophantha*, the use of dry heat to break PY generally does not result in high germination percentages (Table 1).

The seeds of *P. lophantha* appeared to have a very thick testa for the size of the seed. Both Dell (1980) and the present study found that the testa was between 51% and 54% of the seed mass and, in this study, 47% of seed volume. While it appears that relatively few studies have assessed the relative proportions (either mass or volume) of testa and embryo, Lush and Evans (1980) published values for 21 species of grain legumes, including wild and cultivated subspecies/varieties, with and without PY. They found the testa averaged 15% (median 12%) of the whole seed weight and ranged from 3% to 39%. This legume data would indicate that an approximate 50:50 split between testa and embryo is an unusually large investment in the testa.

As noted, Vassal (1971) studied seed structure in 127 species of acacias, both Australian and African. He investigated, amongst many characters, the ratio of seed length to testa thickness. Individual species values for testa thickness were not given. He divided the ratios into four classes: I < 20, II 21–35, III 36–50 and IV 51–65. Of the five taxonomic divisions investigated by Vassal, only two (Phyllodineae – Australian and Gummiferae – African) had species in Class I. Two African species from Class I were illustrated, with line drawings of seed cross sections of *A. victoriae* (fig. 36) and *A. nilotica* (fig. 40). Both drawings show a very thick testa. *P. lophantha* would be in class 1, with a ratio of 16.8. This ratio was calculated for the 51 *Acacia* species in Burrows et al. (2018), but values for individual species were not given. Only two of these 51 species were in Class I – *A. grasbyi* (16.8) and *A. inaequiloba* (19.9) (G. E. Burrows, unpublished data). Thus, *P. lophantha* has a thick testa that represents a large percentage of the seed mass, but several similar species have been recorded, especially in the African acacias.

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In control seeds, the mass and volume of the testa and embryo were almost equal, although the MC of the embryo (6.9%) was about 40% less than that of the testa (11.5%). With full imbibition, the volume and mass of the embryo increased more than the testa as the embryo has a lower MC to begin with and the numerous cotyledon cells (that make up most of the embryo) absorb water by osmotic forces to begin the germination process. Nonetheless, the testa absorbs considerable moisture during imbibition, as shown by going from black to brown as volume increases and going from mechanically strong to weak enough that the embryo can easily expand and the radicle can emerge. Dell (1980) noted that in the early stages of imbibition (first few hours) a gap was present between the testa and embryo, as initially the testa takes up more water than the embryo.

As noted, the seeds made a clear 'click' immediately they were immersed in water at 90–100°C. This also occurred with the Bunsen burner flame, but the 'click' was not as loud. This would appear to be the first time a sound associated with lens popping has been noted. The creation of a sound indicates that detachment is very rapid. This was checked in the two Class I (as described earlier) *Acacia* species (*A. grasbyi* and *A. inaequiloba*) examined by Burrows et al. (2018). No clicking occurred when seeds of these two species were plunged into BW (G. E. Burrows, unpublished data).

Burrows et al. (2019) found that in many of the investigated 48 species of *Acacia*, after PY had been broken and the lens popped, substantial variation in the start of imbibition occurred. Some seeds in a population imbibed within days, while others could take several weeks before imbibition commenced. They termed the popping of the lens a 'fire gauge', with the staggered imbibition a 'rain gauge'. In *P. lophantha*, all seeds with fully popped lenses imbibed promptly and simultaneously. After the EtOH flame treatment, a somewhat staggered imbibition was observed, but this appeared related to incomplete lens popping.

A ring of smooth tissue or cuticle surrounded the funiculus in *P. lophantha* (Figure 2, Dell, 1980). A similar structure was not observed in any of the 51 species of *Acacia* examined by Burrows et al. (2018) (G. E. Burrows, unpublished data).

In laboratory-based germination experiments, seeds are routinely placed on top of a moist medium, usually filter paper. This study shows this is not always optimal, especially when dealing with PY seeds where the water gap may be some distance from the seed's point of contact with the moist substrate. While all the 'on top' seeds had imbibed and 85% had germinated after 72 h, they were, on average, about 30 h slower to full imbibition than the seeds under one layer. As noted, the seeds under paper imbibed more quickly, and this technique could be closer to what seeds experience in moist soil. The seeds submerged in water imbibed quickly (130% vs 40% increase in mass for one layer seeds after 24 h), but their germination, as assessed by radicle extension, was relatively slow, probably related to oxygen levels.

The graded series of HW treatments and times in BW revealed that the lens responded to the severity of the dormancy-breaking treatment, forming mounds at lower temperatures and very short exposures to higher temperatures, to complete lens detachment at higher temperatures and/or longer exposures. This is possibly the first time this correlation has been noted. Three explanations are possible: (i) many studies of legume PY use a single high-intensity HW or BW treatment, rather than a graded series, (ii) length of the HW or BW treatment is usually longer than is necessary and (iii) in few studies of PY do researchers investigate what effect a treatment has had on the lens. This study showed that as long as the lens was visibly popped then water could enter the seed, although, on average, imbibition and germination were slightly quicker when a disk detached, as compared to a mound.

As per the present study, several studies have reported high viability and high levels of PY in seeds of *P. lophantha* (Table 1). Perhaps the most interesting difference between these studies was the seed mass, especially as reported by Harris et al. (2017). Their CSA seeds were, on average, over three times the mass of their WA seeds (about 90 and 27 mg, respectively). Both of their collections were from 7–9 sites/populations and 30–38 individuals (i.e. about four individuals per site). The studies of Bell et al. (1995) (average seed weight 77 mg), Dell (1980) (c. 65 mg) and the present study (c. 70 mg) all used WA seeds. These much lighter WA seeds sampled by Harris et al. (2017) were probably much smaller, with a thinner testa and palisade cells. All these features may have resulted in quite different rates of insect predation, imbibition, germination and seedling performance. Harris et al. (2017) noted their WA seeds had a much higher predation and much lower viability than the CSA seeds.

In conclusion, population variation in features relating to seed size, morphology, viability, degree of dormancy and response to PY-breaking treatments is extensive in *P. lophantha* (Table 1). Thus, not all findings of this study will directly apply to all populations of the species. Nonetheless, compared to most investigated species of *Acacia*, the seeds of *Paraserianthes* are similar in having high levels of PY, a testa composed of three main layers and a type II (simple) lens gap. The two genera differ in that *P. lophantha* has several distinguishing features: (i) the seeds are relatively large and heavy, (ii) the testa makes up a large percentage of the seed mass, (iii) no aril is present, (iv) the macrosclereids are over 150 µm long, (v) with a short BW treatment the lens completely detaches and (vi) a popping lens can produce a relatively loud 'click'.

Acknowledgements. I thank the Charles Sturt University Quantitative Consulting Unit for assistance with the data analysis. I thank Mark Filmer for editing assistance. I thank Aude Gourieroux for helpful comments on the manuscript.

Funding statement. No funding was received for this study.

Competing interests. None declared.

References

Agresti A and Coull BA (1998) Approximate is better than "exact" for interval estimation of binomial proportions. *American Statistician* **52**, 119–126.

Azani N, Babineau M and Bailey CD and **LPWG** (2017) A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny. *Taxon* **66**(1), 44–77.

Bell DT, Plummer JA and Taylor SK (1993) Seed germination ecology in southwestern Western Australia. *Botanical Review* **59**(1), 24–73.

Bell DT, Rokich DP, McChesney CJ and Plummer JA (1995) Effects of temperature, light and gibberellic acid on the germination of seeds of 43 species native to Western Australia. *Journal of Vegetation Science* **6**(6), 797–806.

Brown GK, James EA, Simmons CL and Ahrens CW (2020) Recently naturalized *Paraserianthes lophantha* subsp. *lophantha* displays contrasting genetic diversity and climate relationships compared to native populations. *Diversity-Basel* **12**, 422.

Brown GK, Murphy DJ and Ladiges PY (2011) Relationships of the Australo-Malesian genus *Paraserianthes* (Mimosoideae: Leguminosae LPWG (Legume Phylogeny Working Group) (2007)) identifies the sister group of *Acacia sensu stricto* and two biogeographical tracks. *Cladistics* **27**(4), 380–390.

Bruneau A, Queiroz LPD, Ringelberg JJ, Borges LM, Bortoluzzi RLD, Brown GK, Cardoso D, Clark RP, Conceicao AD, Cota MMT,

https://doi.org/10.1017/S0960258525100020 Published online by Cambridge University Press

Demeulenaere E, Stefano RDD, Ebinger JE, Ferm J, Fonseca-Cortes A, Gagnon E, Grether R, Guerra E, Haston E, Herendeen PS, Hernandez HM, Hopkins HCF, Huamantupa-Chuquimaco I, Hughes CE, Ickert-Bond SM, Iganci J, Koenen EJM, Lewis GP, Lima HCD, Lima AGD, Luckow M, Marazzi B, Maslin BR, Morales M, Morim MP, Murphy DJ, O'Donnell SA, Oliveira FG, Oliveira ACD, Rando JG, Ribeiro PG, Ribeiro CL, Santos FD, Seigler DS, Silva GSD, Simon MF, Soares MVB and Terra V (2024) Advances in legume systematics 14. Classification of Caesalpinioideae. Part 2: higher-level classification. *PhytoKeys* **240**, 1–552.

Burrows GE (2024) Determining the moisture content of *Acacia* seeds. *Australian Journal of Botany* **72**(5), BT24021.

Burrows GE, Alden R and Robinson WA (2018) The lens in focus - lens structure in seeds of 51 Australian *Acacia* species and its implications for imbibition and germination. *Australian Journal of Botany* **66**(5), 398–413.

Burrows GE, Alden R and Robinson WA (2019) Markedly different patterns of imbibition in seeds of 48 *Acacia* species. *Seed Science Research* **29**(4), 270–282.

Burrows GE, Virgona JM and Heady RD (2009) Effect of boiling water, seed coat structure and provenance on the germination of *Acacia melanoxylon* seeds. *Australian Journal of Botany* **57**, 139–147.

Cavanagh T (1987a) Germination of hard-seeded species (Order Fabales). In Langkamp P (ed), *Germination of Australian Native Plant Seed*. Melbourne: Inkata Press, pp. 58–70.

Cavanagh T (1987b) Germination of hard-seeded species: mimosoid seeds (Appendix 4B). In Langkamp P (ed), *Germination of Australian Native Plant Seed*. Melbourne: Inkata Press, pp. 202–206.

Channing KM (2011) *Invasive Populations of Paraserianthes lophantha in Auckland, New Zealand: Distribution, Seed Bank and Methods for Control*. Auckland: University of Auckland.

Crawley MJ (2013) *The R Book. 2nd Edition*. UK: Wiley.

Cromer EL (2007) *Seed Germination and Research Records from Alcoa's Marrinup Nursery. Research Note No. 27*. Alcoa World Alumina Australia.

Dell B (1980) Structure and function of the strophiolar plug in seeds of *Albizia lophantha*. *American Journal of Botany* **67**, 556–563.

Fernández YA, Marrero DF and Martínez-Montero ME (2021) Liquid nitrogen as promotor of seeds germination and seedling growth in tropical legumes. *INGE CUC* **17**(2), 1–10.

Gama-Arachchige NS, Baskin JM, Geneve RL and Baskin CC (2013) Identification and characterization of ten new water gaps in seeds and fruits with physical dormancy and classification of water-gap complexes. *Annals of Botany* **112**, 69–84.

García-Duro J, Cruz O, Casal M and Reyes O (2019) Fire as driver of the expansion of *Paraserianthes lophantha* (Willd.) I. C. Nielsen in SW Europe. *Biological Invasions* **21**(4), 1427–1438.

Geneve RL, Baskin CC, Baskin JM, Jayasuriya KMGG and Gama-Arachchige NS (2018) Functional morpho-anatomy of water-gap complexes in physically dormant seed. *Seed Science Research* **28**, 186–191.

Gunn CR (1984) *Fruits and Seeds of Genera in the Subfamily Mimosoideae (Fabaceae). Technical Bulletin Number 1681*. United States Department of Agriculture, Agricultural Research Service.

Hanna, P. J. (1984) Anatomical features of the seed coat of Acacia kempeana (Mueller) which relate to increased germination rate induced by heat treatment. *New Phytologist* **96**, 23-29.

Harris CJ, Manea A, Moles AT, Murray BR and Leishman MR (2017) Differences in life-cycle stage components between native and introduced ranges of five woody Fabaceae species. *Austral Ecology* **42**(4), 404–413.

Leino MW and Edqvist J (2010) Germination of 151-year old *Acacia* spp. seeds. *Genetic Resources and Crop Evolution* **57**(5), 741–746.

Lenth RV (2024) *Emmeans: Estimated Marginal Means, Aka Least-squares Means*. R Package Version, 1.10.5–0900001.

Lersten NR, Gunn CR and Brubaker CL (1992) *Comparative Morphology of the Lens on Legume (Fabaceae) Seeds, with Emphasis on Species in Subfamilies Caesalpinioideae and Mimosoideae. Technical Bulletin Number 1791*. United States Department of Agriculture, Agricultural Research Service.

Lush WM and Evans LT (1980) The seed coats of cowpeas and other grain legumes: structure in relation to function. *Field Crops Research* **3**(3), 267–286.

McDowell CR and Moll EJ (1981) Studies of seed germination and seedling competition in *Virgilia oroboides* (Berg.) Salter, *Albizia lophantha* (Willd.) Benth. and *Acacia longifolia* (Andr.) Willd. *Journal of South African Botany* **47**, 653–685.

Murphy DJ and Maslin B (2023) *Acacia*: taxonomy and phylogeny. In Wattles: Australian *Acacia* species around the world. In Richardson DM, Le Roux JJ and Marchante E (eds), *CAB International* , pp. 27–40. Boston.

R Core Team (2024) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Salomao AN (2002) Tropical seed species' responses to liquid nitrogen exposure. *Brazilian Journal of Plant Physiology* **14**, 133–138.

Sugii N (2003) Flaming Fabaceae - using an alcohol flame to break seed dormancy. *Native Plants Journal* **4**, 46–47.

Vassal J (1971) Contribution à l'étude morphologique des graines d'*Acacia*. *Bulletin de la Société d'Histoire Naturelle de Toulouse* **107**(1-2), 191–246.

Warton DI and Hui FKC (2011) The arcsine is asinine: the analysis of proportions in ecology. *Ecology* **92**, 3–10.

Yang Y, Jaganathan GK, Biddick M, Li J and Liu B (2020) Identification of the water-gap in *Albizia julibrissim* (Fabaceae: Mimosoideae) following wet and dry heat. *Plant Biosystems* **154**(4), 578–583.

https://doi.org/10.1017/S0960258525100020 Published online by Cambridge University Press