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Effect of boiling water, seed coat structure and provenance on the germination of *Acacia melanoxylon* seeds

Geoffrey E. Burrows[A,D], *James M. Virgona*[B] *and Roger D. Heady*[C]

[A]Institute for Land, Water and Society, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia.
[B]EH Graham Centre for Agricultural Innovation, Charles Sturt University, Locked Bag 588, Wagga Wagga, NSW 2678, Australia.
[C]Fenner School of Environment and Society, The Australian National University, Canberra, ACT 0200, Australia.
[D]Corresponding author. Email: gburrows@csu.edu.au

Abstract. *Acacia melanoxylon* (Mimosoideae or Mimosaceae) is a high quality timber tree with an extensive natural distribution in Australia and a wide genetic and phenotypic diversity. Seeds from three widely differing provenances in Tasmania were tested to determine whether they had different responses to various dormancy-breaking treatments. All provenances had limited germination (<11%) if seeds were untreated and between 85% and 91% germination after 40 days if the seeds were nicked. For all provenances short (≤60 s) exposure to boiling water gave high germination percentages. These values were generally lower, although usually not significantly so, than the germination percentages following nicking. Germination percentages decreased with increasing time of exposure to boiling water, although one provenance had a significantly greater tolerance to one of the longer (20 min) treatments. Nicked seeds germinated quickly and uniformly, whereas those subjected to the boiling-water treatments germinated after a longer period and more gradually. In untreated seeds, the lens was a low, elliptically shaped dome (~110–135 μm wide, 140–190 μm long). In more than 99% of the seeds examined, the structure of the lens was markedly altered after a 10-s exposure to boiling water. A wide diversity of altered lens structure was found, from a circular hole between the macrosclereids, to a short fissure where the macrosclereids did not separate to their bases. Nicked seeds had a 200–375 times greater area for water uptake than a fully disrupted lens and this was probably the principal reason why the nicked seeds germinated sooner and more rapidly.

Introduction

Most *Acacia* species produce seeds with physical dormancy, with the seed coat being the main barrier to water uptake (Baskin and Baskin 1998). The main external feature of the seed coat of most *Acacia* species is the hilum (the scar from the detachment of the funiculus – the vascular supply to the ovule) and on either side of the hilum are the micropyle (prior entry point for the pollen tube) and the lens (also referred to as the strophiole) (Cavanagh 1987; Manning and van Staden 1987). Most of the seed coat comprises a relatively thick cuticle, a palisade epidermal layer of thick-walled radially elongated cells (malpighian or macrosclereid cells) and inner osteosclereids and mesophyll cells (Cavanagh 1987). It is generally considered that the cuticle and mesophyll layers are water permeable and the macrosclereid cells are the main barrier to water movement (Cavanagh 1987; Baskin and Baskin 1998; Morrison *et al*. 1998; Baskin *et al*. 2000). The lens is considered the main area of weakness in the palisade layer (Morrison *et al*. 1998; Baskin *et al*. 2000), although Brown *et al.* (1969) reported that boiling-water treatments caused widespread breaks in the palisade layer. At the lens, the macrosclereid cells are usually shorter than elsewhere in the seed coat and are attached to thin-walled cells in the outer mesophyll (Dell 1980; Hanna 1984; Cavanagh 1987).

The role of fire in breaking the seed dormancy in *Acacia* is well known (e.g. Tozer 1998). Baskin and Baskin (2000) evaluated the role of soil microbes and abrasion by soil particles in the breaking of physical dormancy in nature. In nursery production the seed coat can be damaged in non-specific locations with treatments such as immersion in sulfuric acid or being tumbled with sharp sand, or in specific locations by nicking or immersion in hot or boiling water. Nicking is used to damage the seed coat at a selected location, whereas boiling water disrupts the structure of the lens (Dell 1980; Hanna 1984; Cavanagh 1987). Of all these treatments and others (e.g. hot wire, fire, dry heat, microwaving) boiling water is perhaps the easiest method to apply consistently to bulk lots of seeds, with no pronounced safety issues. However, few studies have examined the influence of a wide range of time of exposure to boiling water on the germination of *Acacia* species.

Acacia melanoxylon R.Br. (Mimosoideae or Mimosaceae) is a small to large tree (6–30 m high) and occurs across an extensive range of soils and climatic conditions (Playford *et al*. 1993). It produces a highly valued timber and has been established in plantations in several countries (Pinkard and Beadle 2002). It occurs along the eastern coast of Australia, from southern Tasmania to northern Queensland, with a western outlier in the Mount Lofty Ranges in South Australia (Farrell and Ashton 1978)

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and has wide genetic variation across its range (Playford *et al*. 1993). Even within individual states of Australia, a wide ecological tolerance is displayed (Farrell and Ashton 1978). Prolific seedling regeneration after fire has been recorded in a rainforest in western Tasmania (Hill 1982). Few studies have examined the influence of provenance on seed germination in *Acacia*. Because *A. melanoxylon* exhibits wide ecological variance, we considered it was a good subject to determine whether any interaction existed between the provenance and boiling-water treatments.

Materials and methods

Seed selection and treatment

Seeds of *A. melanoxylon* were sourced from the Tasmanian Seed Centre, which is managed by Forestry Tasmania. Seeds from three provenances (L08, L16, L21) within Tasmania were obtained (Table 1). These three provenances provide a relatively wide selection of the genetic diversity available in the Tasmanian populations.

Average seed mass for each provenance was measured, on the basis of 20 randomly selected samples of 100 seeds for each provenance. To measure the rate of imbibition, randomly selected samples of 100 seeds for each provenance were assigned to three treatments (control, boiling water for 10 s, nicked), then weighed, placed on moistened filter paper in Petri dishes and placed in an incubator at 25°C. The seeds were then reweighed after surface drying at various intervals across 6 days. The percentage change in fresh mass was calculated.

To determine seed viability, two replicates of 50 randomly selected seeds of each provenance were selected. The seeds were cut in longitudinal section, placed cut surface down on filter paper saturated with 2,3,5-triphenyltetrazolium chloride (1%) at 20°C for 6 h. A pink colouration in the embryo was taken to indicate a seed was viable.

Seeds were subjected to the following treatments:

(1) no treatment;
(2) seeds were placed in 200-mL plastic containers and 150 mL of boiling water was poured over the seeds; the containers were at room temperature on a laboratory bench; the seeds were left in the slowly cooling water for 5 min, after which the hot water (now ~75°C) was poured off and replaced with water at 20°C for 30 min, then drained;
(3) as per (2), except the boiling water was poured over the seeds and left for 20 min (water was ~50°C when poured off);
(4) the seeds were loosely enclosed in a bag of muslin cloth and dipped into 500 mL of boiling water for 2–5 s; the seeds were a small volume compared with the water and did not cause the boiling to cease; after exposure to the boiling water they were then placed in water at 20°C for 30 min, then drained;
(5) as per (4), except the seeds were exposed to boiling water for 10 s;
(6) as per (4), except the seeds were exposed to boiling water for 1 min;
(7) as per (4), except the seeds were exposed to boiling water for 5 min;
(8) as per (4), except the seeds were exposed to boiling water for 20 min;
(9) as per (4), except the seeds were exposed to boiling water for 60 min; and
(10) a small nick, to the level of the outside of the cotyledons (Fig. 1*I*), was made in the distal rounded end (away from the radicle and epicotyl) of each seed.

Table 1. Geographic and climatological information for the three selected Tasmanian provenances of *Acacia melanoxylon*, as sourced from the Tasmanian Seed Centre

Provenance code	Location	Annual rainfall (mm)	Minimum temperature (°C)	Altitude (m)
L08	NE Tasmania	900–1200	−2 to −5	<300
L16	Derwent Valley	<650	−5 to −10	<300
L21	Central West Coast Lowlands	>1600	−5 to −10	<300

Each treatment of each provenance was repeated twice, i.e. two lots of 100 seeds of each provenance were subjected to each treatment. In total, 6000 seeds were treated. Six separate hot plates and containers of boiling water were used, and seeds from the one replication of a provenance were treated in the same container of boiling water.

Once treated, the 100 seeds of a treatment were separated into four lots of 25 seeds. The 25 seeds of each of the 10 treatments were planted into randomly allocated rows within a 35 cm long × 29 cm wide × 6 cm deep germination tray. The trays were 80% filled with a commercially available germination mix, and the seeds were buried to a depth of ~5 mm. Each replicate of a provenance consisted of four trays, each with 10 rows of 25 seeds. Seeds were sown within 2 h of the treatment.

The trays were placed on bottom-heated benches in a fogged glasshouse. The temperature of the mix at the level of the seeds was maintained at an average of 22°C, and ranged between 18°C some mornings and 26°C some afternoons. The experiment was usually assessed at 3–4-day intervals, and a seed was recorded as germinated when the hypocotyl or cotyledons emerged through the mix. The experiment ran for 40 days. Seed-raising mix and glasshouse conditions were used, rather than filter paper and incubators, so the results would have a greater applicability to commercial nursery operations.

Microscopy

Seed length, width and thickness were measured for 10 untreated seeds per provenance under a dissecting microscope. Lens length and width and seed-coat thickness, including cuticle, macrosclereid and mesophyll depth, were measured on 10 untreated seeds per provenance with a scanning electron microscope (SEM) and image analysis software. The seed-coat features were measured on the flattish sides of the seeds, midway between the proximal and distal ends, after fracturing the seeds to expose the coat in transverse section.

In all, 100 seeds of each provenance were subjected to Treatment 5 (10 s in boiling water), air-dried, then the lens was examined under a dissecting microscope. The lens was assessed for its shape as per the four or five basic shapes shown in Fig. 1. To visualise the shape of the 'popped' or altered lens better, two seeds

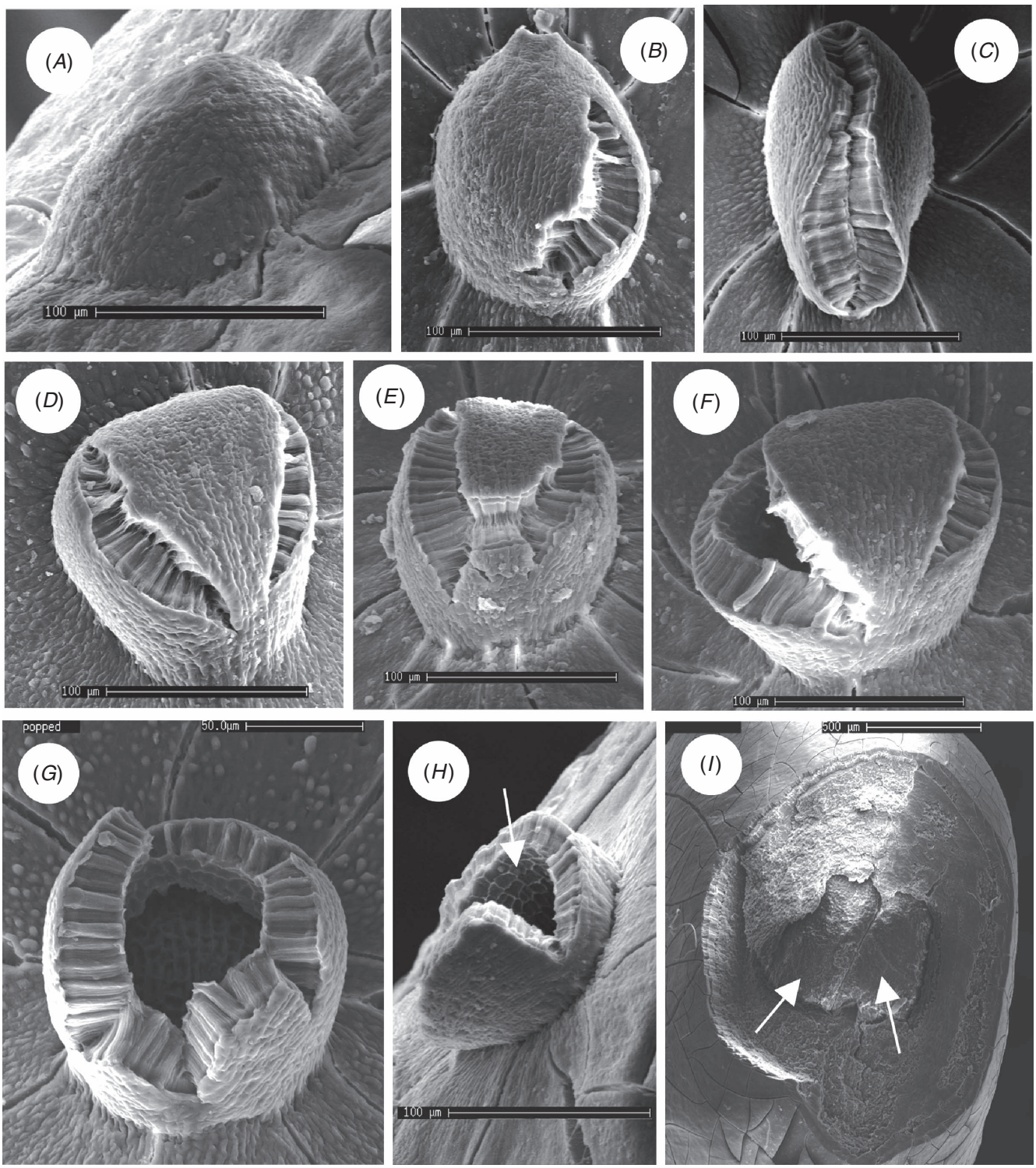

Fig. 1. Scanning electron microscopy images of seeds of *Acacia melanoxylon*, (*A–H*) after a 10-s exposure to boiling water, showing differing lens structures, or (*I*) after being nicked. (*A*) The surface of the lens had formed a hemispherical shape and no major cracks were present in its surface. (*B–F*) Images where the macrosclereid cells of the lens had at least partially separated, with a distinct gap present only in *F*. (*G*) Lens that had completely 'popped' after a 10-s exposure to boiling water. Note the 70–80-µm-diameter hole for water to enter the seed. Note also the shorter macrosclereid cells to the top of the image (away from the hilum). (*H*) Similar to *G*, except showing the tops of the thin-walled cells (arrowed) that were ruptured when the lens popped. (*I*) Nicked seed showing the tips of the cotyledons (arrowed).

from each of the basic shapes from each of the three provenances were examined with a SEM.

For semi-thin sections, the normal seed coat and the lens region from three untreated seeds of provenance L08 were fixed in 50% formalin–acetic acid–alcohol, dehydrated through a graded ethanol series and infiltrated with Leica HistoResin® (Leica Microsystems, Heidelberg, Germany) under a slight vacuum for a minimum of 2 days. The samples were placed in pharmaceutical gelatin capsules containing HistoResin® and polymerised overnight at 60°C. They were then longitudinally sectioned at 4 µm with tungsten carbide-tipped steel blades fitted to a motorised retraction microtome. Sections were stained with 0.5% toluidine blue and observed under bright-field microscopy.

Statistical analysis

A two-way analysis of variance was performed on the percentage germination data, with provenance and seed treatment as factors, each replicated twice. Before analysis, raw germination percentages were adjusted for each provenance on the basis of seed viability derived from the tetrazolium results. The proportions of viable seed were 97%, 94% and 83% for L08, L16 and L21, respectively. For the seed morphology and anatomy characteristics, a 1-way ANOVA was carried out and comparisons with least significant differences are presented. For both analyses, performed with GENSTAT® Release 10 (VSN International Ltd, Oxford), the residuals were tested for normality and transformation was not required.

Results

Seed dimensions, mass and structure

The seeds of L08 were significantly heavier, wider and longer than those of the other two provenances (Table 2). The seeds of L21 were significantly lighter and thinner than those of the other two provenances (Table 2).

Most seeds examined with a dissecting microscope had a network of major cracks in the cuticle. It was not clear whether the network of fine cracks in the surface of the cuticle (Fig. 2) was an artefact of SEM preparation. SEM examination of the lens of control seeds of all provenances revealed a low elliptical mound (Table 3), close to the hilum and on the opposite side of the hilum to the micropyle (Fig. 2). Although most of the seed coat was smooth, the surface of the lens had a fine sculpturing (Fig. 2*D*). Examination of the seed coat in tangential section showed a relatively thick cuticle (Table 3), densely packed narrow-diameter macrosclereid cells perpendicular to the seed surface and then the mesophyll cells, mainly parallel to the seed surface, with some apparently shorter cells on the inner and outer surfaces of the mesophyll (Fig. 3*A–D*).

Table 2. Average seed mass (100 seeds) and seed dimensions (10 seeds) for three provenances of *Acacia melanoxylon*

l.s.d., $P = 0.05$

Provenance	Av. mass100 seeds (g)	Av. seed width (mm)	Av. seed length (mm)	Av. seed thickness (mm)
L08	1.80	2.96	4.80	1.49
L16	1.34	2.62	4.19	1.36
L21	1.20	2.50	4.07	1.17
l.s.d.	0.11	0.16	0.31	0.14

Light microscopy indicated that at the lens, the cuticle was very thin, the macrosclereid cells were shorter than measured elsewhere (Table 3) and were progressively shorter away from the hilum (Figs 1*G*, 3*E*). The macrosclereids of the lens were attached to a layer of thin-walled cells and the vascular tissues from the hilum curved close to the lens region (Fig. 3*F*).

Examination of the lens of seeds that had been exposed to boiling water for 10 s revealed a wide diversity of structural change (Fig. 1*A–H*). In >99.5% of seeds, there had been some obvious change in lens morphology. In 50–60% of seeds of all three provenances, the macrosclereids of the lens had split along their full length and there was a gap between the bases of the macrosclereids (Fig. 1*F–H*). In 40–50% of seeds, the macrosclereids had split although there was no obvious gap (Fig. 1*B–E*). Note that in Figs 1*G* and 3*E*, *F*, the macrosclereids of the lens were longest near the hilum. In seeds with a fully popped lens (e.g. Fig. 1*G*, *H*), the average internal diameter of a popped lens was 73 µm, with an average area of the opening of ~0.0042 mm^2. In the nicked seeds (Fig. 1*I*), the area to the inside of the macrosclereids averaged 1.27 mm^2, which was ~300 times greater than that for a fully popped lens.

Imbibition

The nicked seeds for all three provenances doubled their fresh mass within 6 h of the start of imbibition, and seeds of all provenances reached a maximum increase in fresh mass of ~150% after 24 h. After 48 h, control seeds of all provenances had increased in fresh weight only by 1–5%, whereas boiling water-treated seeds of L08, L16 and L21 had increased by 1.4, 11.1 and 25%, respectively.

Germination

All three provenances produced low (5–10%) germination percentages from untreated seeds (Table 4). Nicking usually produced the highest germination percentages, although nicked seeds were not statistically different from the best of the boiling-water treatments. All provenances exhibited significantly greater germination than did the control when seeds were exposed to boiling water for 5 min or less, or exposed to boiling water that was allowed to gradually cool (Table 4).

Within the provenances, there was no statistical difference in germination percentages with short exposures to boiling water (2–5 to 60 s) (Table 4). Increasing the exposure to boiling water from 1 to 5 min produced a significant decrease in germination percentage for all provenances. With further increases in the length of time of exposure to boiling water, germination percentages further decreased. This was particularly pronounced in L21 (Table 4). Seeds of L08 showed a greater tolerance to extended exposure to boiling water than did those of the other two provenances, with significantly greater germination than for the other provenances after a 20-min exposure to boiling water; 10% of the seeds germinated even after 1-h exposure to boiling water.

The nicked seeds germinated sooner and at a greater rate than those in any of the other treatments (Fig. 4). For all provenances, more than 90% of final germination was recorded after 11 days. The provenances exhibited different germination rates in

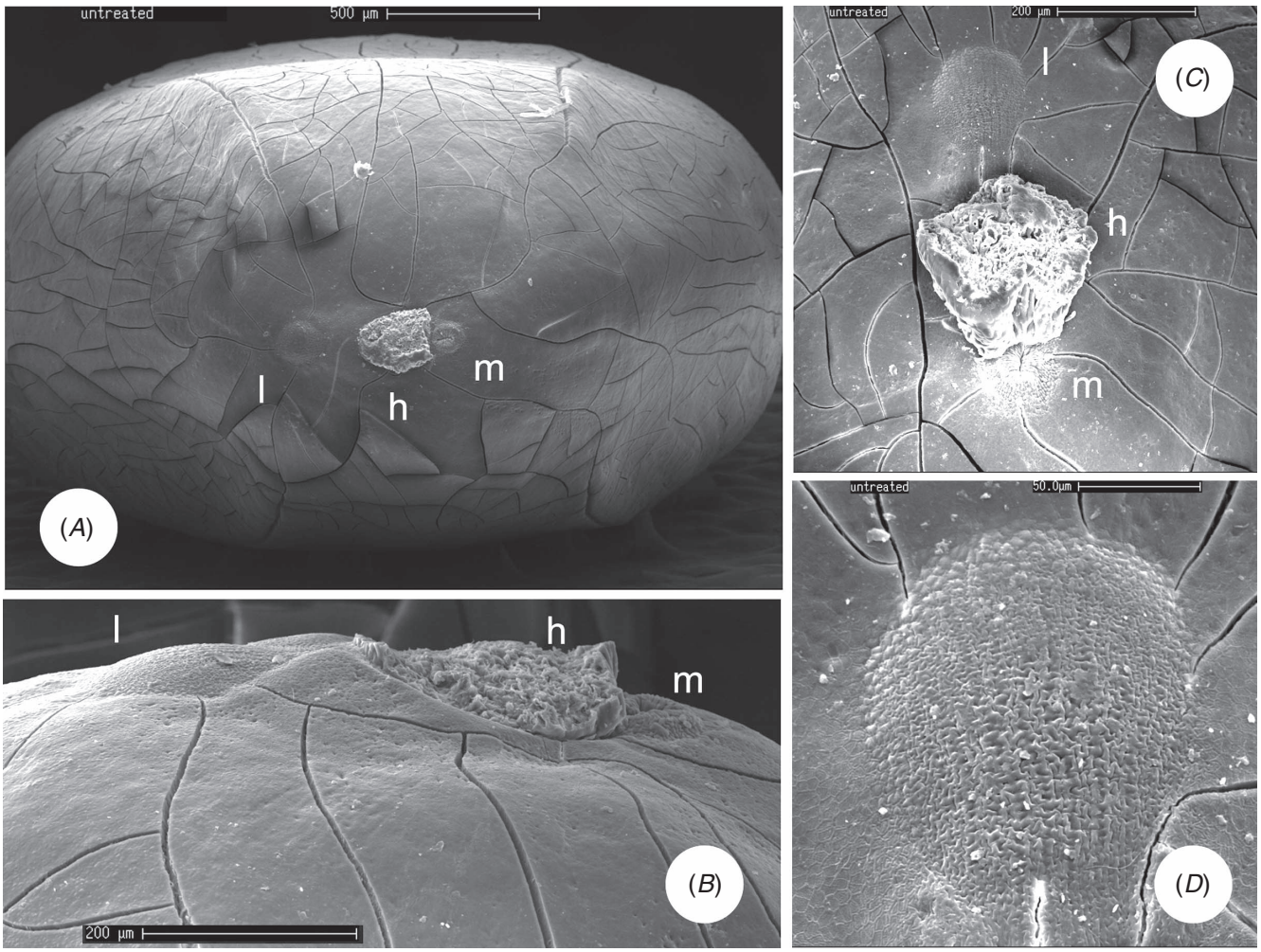

Fig. 2. Scanning electron microscopy images of untreated seeds of *Acacia melanoxylon*. (*A*) Proximal end of seed, showing the lens (l), hilum and attached funicular tissues (h) and micropyle (m). Note the small size of the lens compared with that of the seed. Note also the numerous fine cracks in the otherwise smooth cuticle. (*B*) Side view of the lens, hilum and micropyle. (*C*) Detailed view of the lens, hilum and micropyle. (*D*) Detail of lens, showing it was a slightly raised elliptical mound and that a fine sculpturing was present on its surface, unlike most of the remainder of the seed surface.

Table 3. Various average seed coat and lens dimensions (in µm) for three provenances of *Acacia melanoxylon*
MS = macrosclereid: l.s.d. at $P = 0.05$: n.s., not significant

Provenance	Cuticle depth: normal	Cuticle depth: lens	MS depth: normal	Range MS depth: lens	Mesophyll depth	Seed coat depth total	Lens width	Lens length
L08	26.1	<2	47.9	14–31	115.6	189.6	133.7	188.3
L16	25.4	<2	43.4	15–31	108.6	177.3	109.3	142.3
L21	24.3	<2	42.7	14–30	102.7	169.7	113.9	148.3
l.s.d.	n.s.		3.1		n.s.	13.9	10.3	14.0

response to the boiling-water treatments. Seeds of L21 receiving Treatments 2–6 reached ~75–85% of their final germination after 18 days, and there was a <25% increase in germination during the final 15 days of recording (Fig. 4). In contrast, seeds of L08 receiving Treatments 2–6 reached <40% of their final germination by Day 18 (Fig. 4). Seeds continued to germinate relatively evenly between Days 18 and 40 (Fig. 4), and it is probable that they would have continued to germinate after Day 40. The response of L16 was intermediate (Fig. 4).

Although the results are not presented here, this experiment was repeated 2 years later with the same seed batches that had been stored at room temperature. The same basic results (final germination percentages, relative differences among the provenances, germination rates) were again obtained.

Discussion

Various authors have reported on the influence of boiling water on the germination of *A. melanoxylon* (Gupta and Thapliyal 1974;

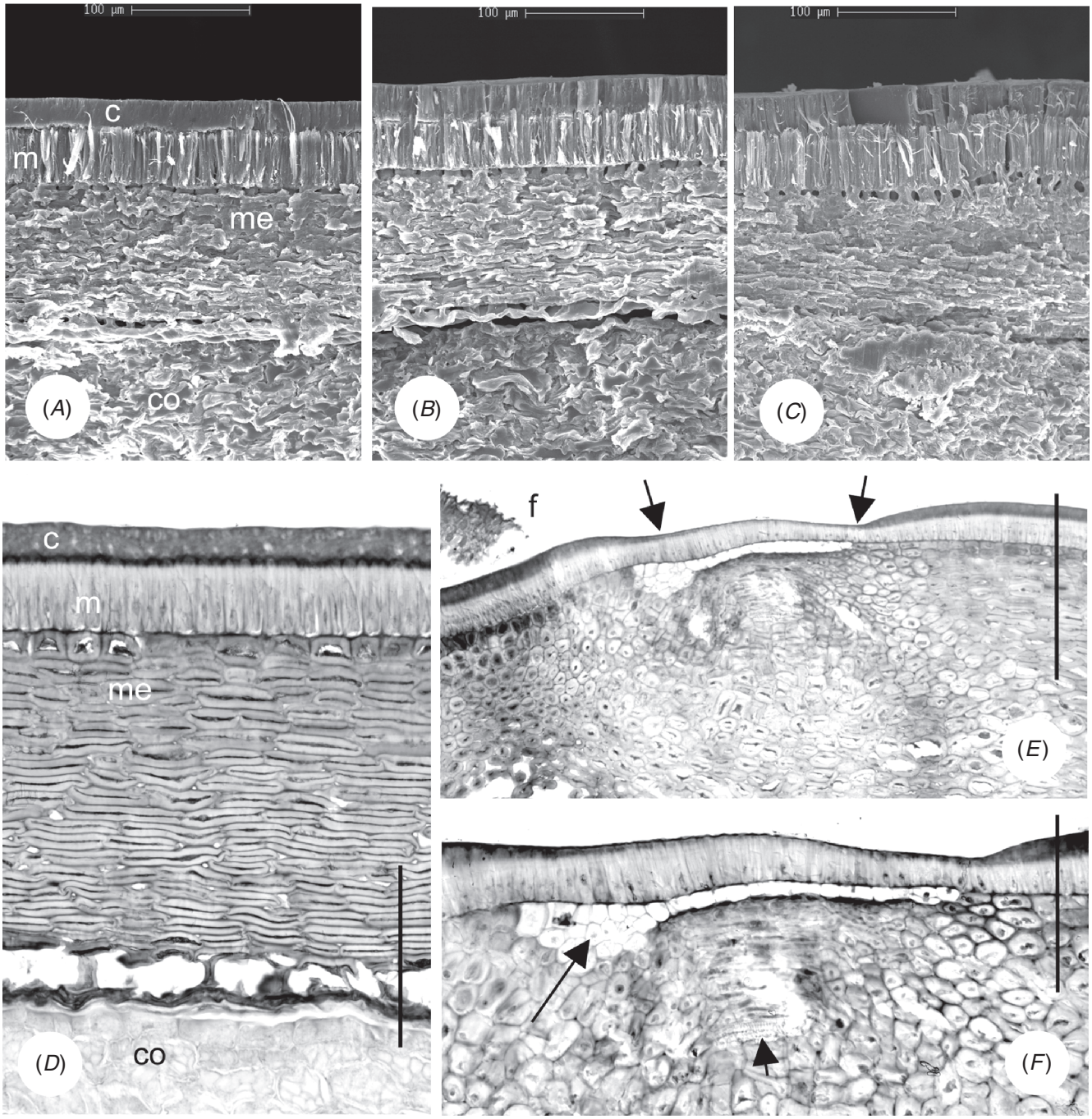

Fig. 3. Images of the seed coat of *Acacia melanoxylon*, cut or fractured, in cross-section. (*A–C*) Scanning electron microscopy images of provenances L08, L16 and L21, respectively. c, cuticle; m, macrosclereids; me, mesophyll; co, cotyledon. (*D*) Light microscopy image of provenance L08. Scale bar = 100 µm. (*E*) Section of the seed coat showing the structure of the lens (between the arrows). Note that the palisade cells of the lens were shorter than elsewhere in the seed coat and were covered by a much thinner cuticle. f, edge of the funiculus. Scale bar = 200 µm. (*F*) Detail of an area close to that shown in *E*. Note the thin-walled cells (longer arrow) beneath the macrosclereid cells of the lens. The shorter arrow indicates some of the xylem cells that were in close proximity to the lens. Scale bar = 100 µm.

de Zwaan 1978; Farrell and Ashton 1978; Bell and Bellairs 1992; Menzies *et al*. 1993) although not in great detail. Gupta and Thapliyal (1974) tested only two boiling-water treatments, with the better treatment (50% germination) apparently being very similar to Treatment 2 of our study. De Zwaan (1978) tested only one boiling-water treatment (3 min) and recorded 98% and 2% germination for 'good-' and 'poor-quality' seeds, respectively. Farrell and Ashton (1978) tested 10 durations of boiling-water treatment, including six treatments of 1 min or less; however, their only recorded finding was that 30-s treatment with boiling water

Table 4. Average germination percentages for seed from three provenances (L08, L16, L21) of *Acacia melanoxylon* subjected to various boiling-water (BW) and nicking treatments, 40 days after treatment

l.s.d. = 13 (at $P = 0.05$)

Treatment	Provenance		
	L08	L16	L21
1. Control	9	10	6
2. 5-min BW soak	85	79	91
3. 20-min BW soak	87	82	93
4. BW 2–5 s	84	78	87
5. BW 10 s	76	74	90
6. BW 1 min	79	67	89
7. BW 5 min	57	36	51
8. BW 20 min	41	12	3
9. BW 60 min	10	3	0
10. Nicking	91	85	86

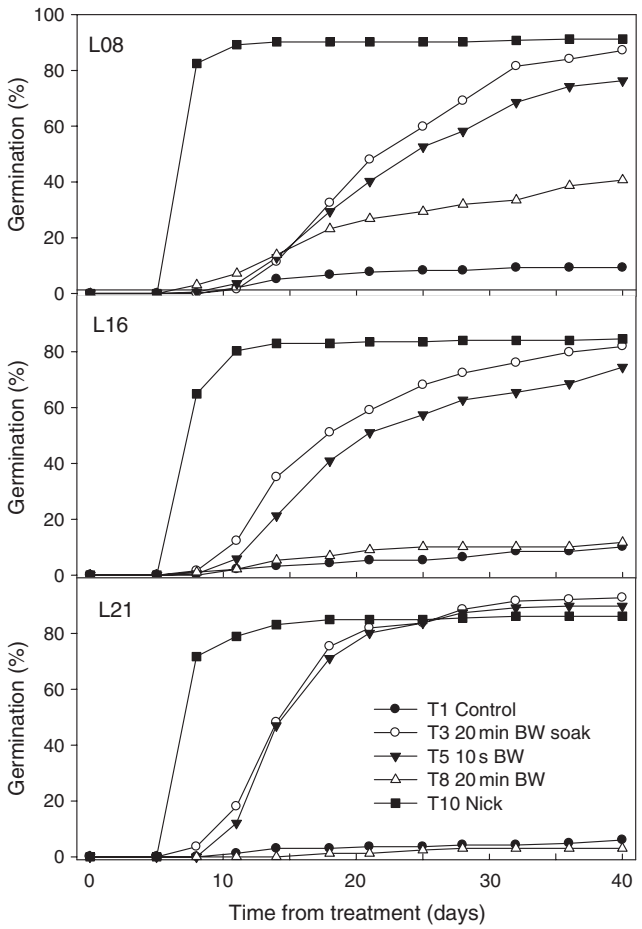

Fig. 4. Accumulative germination percentage for the *Acacia melanoxylon* provenances L08, L16 and L21, across the duration of the experiment, for selected treatments. T, treatment.

was the best after 7 days and one of the better after 30 days. Bell and Bellairs (1992) investigated several *Acacia* species and, with a standard 1-min boiling-water treatment, recorded the best germination of ~50% for *A. melanoxylon*. Menzies *et al*. (1993) reported some apparently inconsistent results on the basis of pouring boiling water on seeds, and then letting the water gradually cool.

The effect of a more comprehensive range of boiling-water treatments has been investigated for a range of other species from the Mimosoideae (e.g. Beadle 1940; Clemens *et al*. 1977; Khasa 1993; Gosling *et al*. 1995; Cervantes *et al*. 1996; Sacheti and Al-Rawahy 1998; Teketay 1998; Rehman *et al*. 1999; Hopkinson and English 2004). As expected, most papers reported a decline in germination percentage with increasing length of boiling-water exposure. In a few studies, the seeds of some species were relatively unaffected by extended periods (10 min or longer) (Clemens *et al*. 1977; Sacheti and Al-Rawahy 1998), whereas those of other species were adversely affected by relatively short exposures (1–3 min) (Clemens *et al*. 1977; Khasa 1993; Gosling *et al*. 1995; Teketay 1998; Rehman *et al*. 1999; Hopkinson and English 2004). The present study supports the conclusion that the briefest exposure to boiling water breaks physical seed-coat dormancy without harmful effects. As Hopkinson and English (2004) noted the change in lens structure is almost instantaneous, whereas the killing effect is progressive. Villiers (1972) noted a standard method for dormancy breaking in *A. melanoxylon* seeds is several minutes in boiling water, whereas the present study indicated this was unnecessarily severe. Tran and Cavanagh (1984) noted that boiling is often considered to 'soften' the seed coat, which may explain why longer than necessary treatments may be used as 'standard'. An understanding of lens structure indicates why very short exposure to boiling water can be effective.

From a perspective of nursery production, the present experiment clearly indicates that short exposure to boiling water, followed either by slow or rapid cooling, resulted in high germination percentages for all three provenances and should be suitable for seeds of *A. melanoxylon* from a wide range of locations. In contrast, Farrell and Ashton (1978) reported a very wide range of germination percentages (10–92, average 49) after 30 days (30-s boiling-water treatment) for 17 Victorian populations of *A. melanoxylon*. This may be related to differences in seed quality between direct collecting of seeds from the bush and purchasing seeds from a commercial supplier.

The three provenances exhibited significant differences in seed size and mass (Table 2), various aspects of seed coat and lens structure (Table 3), germination percentages after longer boiling-water treatments (Table 4) and in germination rates (Fig. 4). Provenance L08 had the highest germination percentages after the longer boiling-water exposure (Table 4) and the slowest germination rates after boiling-water treatments (Fig. 4). In contrast, Provenance L21 had the lowest germination percentages after longer boiling-water exposure and the quickest germination rates after boiling-water treatments. Given that L21 seeds germinated relatively quickly after short-duration boiling-water treatments (Fig. 4), it is possible that water entered the seeds relatively quickly and, consequently, imbibition started relatively rapidly in a large percentage of the seed population. This suggestion is supported by the differences in weight of the imbibing seeds. If this is the case, then in the longer exposures to boiling water it is possible that the embryo (particularly the epicotyl and radicle that are located near the lens)

could be moist-heat injured. The seed weighing experiment showed that after a short boiling-water treatment water entry is greatest into L21 during 24–48 h. It is possible that it is water entry through the lens during the longer boiling-water treatments (20 and 60 min) that leads to reduced germination, especially in L21. It is probable that the moist heat caused the seed mortality as the seeds of some *Acacia* species are still viable after several hours at between 100°C and 105°C dry heat (Cavanagh 1987).

Nicking *Acacia* seeds usually produces the highest final germination percentage and the greatest germination rates (Cavanagh 1987). A fully popped lens of *A. melanoxylon* had an area for water entry of ~0.004–0.005 mm^2 (Fig. 1*G*, *H*), whereas nicking just to expose the tips of the cotyledons produced an area of 1.0–1.5 mm^2, ~200–375 times greater than the area for the fully popped lens (Fig. 1*I*). Nicking leads to more rapid imbibition (150% fresh weight increase in 24 h) and presumably to earlier germination and greater germination rates. Similar to the present study, Clemens *et al*. (1977) and Cavanagh (1980) noted that nicked *Acacia* seeds were fully imbibed after 15–24 h and the increase in fresh mass was from 120–160% (Clemens *et al*. 1977) to 198% (Das and Saha 1999). Imbibed seeds are much larger than the dormant ones (Dell 1980) and Cavanagh (1980) suggested that once some swelling occurred the seed coat may become permeable at multiple sites.

Cavanagh (1980) noted that imbibition of *Acacia* seeds is initially restricted by the small diameter of the lens. He gave a diameter of 0.1 mm for the lens of *A. longifolia*, which is similar to that of *A. melanoxylon*. In *Albizia lophantha*, the lens falls away as a circular 'plug', 250–300 µm in diameter (Dell 1980). This is at least a 10 times greater area than in *A. melanoxylon*. Few illustrations or photographs are available of a 'popped' lens after a hot-water treatment for species from the Mimosoideae (e.g. Dell 1980; Tran 1981; Hanna 1984; Serrato-Valenti *et al*. 1995). Das and Saha (1999) illustrated the appearance of the lens after sandpaper and acid scarification. Lersten *et al*. (1992) provided SEM images of the lens of 29 species from the Caesalpinioideae (13 species) and the Mimosoideae (16 species, nearly 50 images). Although the seeds were essentially untreated in some cases, the lens had completely fallen away during specimen preparation, rather than the cells erupting outwards although remaining attached to the seed as in the present study (Fig. 1). In the Mimosoideae species, the resulting gap (up to 270 µm wide and 600 µm long) was much greater than has been recorded for *Acacia* species. The present study is possibly the first to show that a 'popped' lens of a single *Acacia* species or even a single provenance can have a wide range of morphologies (Fig. 1*A–H*), and the different morphologies can be relatively evenly distributed in a population of boiling water-treated seeds. The rapid and relatively uniform germination of the nicked seeds, compared with the delayed and slower germination of the boiling water-treated seeds, indicated that a 'popped' lens had a reduced rate of imbibition. It is also possible that the range of 'popped'-lens morphologies may be associated with the slower germination, i.e. imbibition may be slower in a lens such as in Fig. 1*B–E* than in Fig. 1*F–H*. However, in the weighing experiment many seeds with a fully opened lens had not swollen after 6 days, even in L21. The slower germination, especially in L08 (Fig. 4), would suggest that some other cause besides water travelling past the macrosclereid layer was delaying germination in many seeds.

The overall seed size was similar to that recorded for the same species by Tran (1979). Seed mass varied significantly among the three provenances (Table 2), with L21 seeds having only 67% of the mass of L08 seeds. Even so, the averages fall within previously recorded values for *A. melanoxylon*. Farrell and Ashton (1978) recorded 0.079–0.207 g for 100 seeds (average 0.140 g) for 17 Victorian populations, although this is almost certainly out by a factor of 10. Menzies *et al*. (1993) used seedlots with 60 500 and 73 300 seeds kg^{-1}, which equates to 1.65 g and 1.36 g per 100 seeds, respectively, whereas Bell and Bellairs (1992) recorded 1.55 g per 100 seeds. The total seed-coat thickness (Table 3) was similar to that recorded for *A. melanoxylon* by Cavanagh (1987) of 160–180 µm and Tran (1981) of 192 µm. However, Cavanagh (1987) recorded macrosclereid cells that had lengths of 60–70 µm and 40–45 µm in the normal seed coat and lens, respectively. This is ~40% longer than that recorded in the present study (Table 3).

Conclusion

Seeds from all three investigated provenances of *A. melanoxylon* had a relatively high average germination (67–90%) after an exposure to a range of relatively short (3–60 s) boiling-water treatments. From a practical perspective, these results indicated that very short exposures to boiling water will break dormancy without decreasing embryo viability and that a relatively wide range of *A. melanoxylon* seeds will respond to these short exposures in a similar manner. Although a uniformity was present in these overall results, there were some significant differences among the provenances in average seed mass, various seed dimensions, response to longer boiling-water treatments and germination rates. Differences in germination rate between the boiling water and nicking treatments correlate well with measurements of the area available for water uptake for imbibition.

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