

Acid Scarification:

an effective method of removing physical dormancy in five Western Australian Acacia species



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Summary

The removal of physical dormancy was investigated in seedlots of five *Acacia* species including three varieties of *A. microbotrya*. Germination following mechanical scarification was not significantly different from the viability level predicted by tetrazolium tests in *A. anthochaera*, *A. cyclops*, *A. lasiocalyx* and *A. microbotrya*. Significantly less germination was recorded in *A. murrayana* and the *A. microbotrya* varieties: *borealis* and *microbotrya* P1 and P3. Compared to manual scarification, wet heat shock treatments were much less effective and, with one exception, dry heat shock treatments for 1–30 min at 90°C, 120°C and 150°C were completely ineffective. By contrast, scarification with concentrated sulphuric acid gave similar results, in most cases, to manual scarification. Although the effect of treatment time varied between species, immersion for 40, 60 or 80 min resulted in highest or near-highest germination in all cases except *A. microbotrya* var. *microbotrya* P3 which germinated best after 100 min treatment.

Introduction

Impermeable seed coats, such as those exhibited by many seeds in the *Fabaceae* family, are of critical importance for species survival (Fenner, 1985). This is particularly true in semi-arid and arid environments where moisture levels may be low and erratic (Quinlivan, 1971). Germination of species with impermeable seed coats can be spread over a number of years in the natural situation and may be attributable to differences in thickness and permeability of the seed coat (Egley, 1989). The environmental conditions under which the seed matures will influence the degree of hard-seededness of the collection. Hard-seededness enables seeds to remain dormant in the soil for long periods of time until environmental conditions are favourable for germination, giving these species the potential to form soil seed bank reserves (Rolston, 1978). Although this spread of germination is desirable in the wild it poses a number of problems for nursery management and for revegetation or rehabilitation programs.

Efforts to reduce hard-seededness aim to improve total seed germination and synchronicity of emergence. Hard-seededness can be broken down by artificial methods that disrupt the barriers on or near the seed surface, induce loosening of the seed coat at weak points, or cause fissures in the seed (Egley, 1989). Such methods include manual or mechanical scarification to forcibly remove a section of the coat. These methods can give reliable results with the final germination often being close to the germination potential of the seed (Cavanagh, 1987a). The disadvantage of manual scarification is that it is very time consuming and not suited to large quantities of seed. Injury during mechanical scarification may also result in decreased viability and vigour.

Thermal shock, involving wet or dry heat, can also release physical dormancy from hard seeded species by causing ruptures in the seed wall thereby allowing imbibition and germination to occur (Egley, 1989; Mayer and Poljakoff-Mayber, 1989). Treatment of seeds with chemicals such as acids can also break hard-seededness simply by corroding away layers of the seed coat (Cavanagh, 1987a). The effectiveness of these treatments is influenced by the temperature (heat shock) and duration (and concentration) of treatment (Cavanagh, 1987a).

This study aimed to determine the most appropriate and effective method for reducing hard-seededness, thereby increasing percent, speed and uniformity of germination in a range of temperate *Acacia* species from Western Australia. Uniformity in germination and subsequent seedling growth is of vital importance for good nursery management. The species (and varieties) chosen for this study are under investigation for use in farm forestry, as possible sources of wood products, gums or tannins, fodder, edible seed crops or solid fuel. The results of this study will assist in the definition of a strategy for large-scale nursery production for these and other *Acacia* species that may be required for commercialisation, restoration or rehabilitation programs.

Materials and Methods

1. Seed Material

Seeds of four *Acacia* species (*Acacia murrayana* F. Muell ex Benth., *A. lasiocalyx* C.R.P. Andrews, *A. antochaera* Maslin and *A. cyclops* G. Don) and three varieties of *A. microbotrya* (*A. microbotrya* Benth., *A. microbotrya* Benth. var. *borealis* E. Pritz., *A. microbotrya* Benth. var. *microbotrya*) were supplied by the Manjimup Seed Centre, Forest Products Commission, Government of Western Australia. All seeds were collected from wild populations in Western Australia between 1999 and 2000. Two populations (P1 and P3) of the variety *A. microbotrya* var. *microbotrya* were provided, enabling the germination potential of eight collections of *Acacia* to be investigated. These seed lots comprised a variety of seed sizes and coat thicknesses.

2. Viability of Seeds

Prior to treatment, seed viability of each collection was assessed using the chemical stain, tetrazolium chloride (International Seed Testing Association, 1999). Four replicates of 25 seeds from each collection were manually scarified, imbibed on plain water agar (10 g l^{-1}) for 48 h, then transferred to a solution of 2,3,5 triphenyl tetrazolium chloride (10 g l^{-1}) for 48 h. Embryos were excised and only seeds where the embryo and most of the cotyledons stained red were deemed to be viable.

3. Seed Germination

Seeds were incubated for 14 d on plain water agar (10 g l⁻¹) held in 9 cm plastic Petri dishes at a constant temperature (15°C) under a 12 h photoperiod. Germination was defined as the emergence of the radicle by at least 2 mm from the seed coat. At the conclusion of all germination treatments, any ungerminated seeds were cut to assess their condition. Firm seeds were deemed potentially viable. Seed numbers for all treatments in the three experiments for *A. murrayana*, *A. lasiocalyx*, *A. anthochaera*, *A. cyclops*, *A. microbotrya*, *A. microbotrya* var. *microbotrya* (P1) consisted of four replicates of 25 seeds. Due to low seed numbers, four replicates of only 15 seeds were used for collections of *A. microbotrya* var. *borealis* and *A. microbotrya* var. *microbotrya* (P3).

4. Manual Scarification, Wet Heat and Control

Incubation of seed after manual scarification was used to determine the maximum potential germination for each collection. Wet heat treatment (soaking in hot (90°C) water for 20 min) provided a comparison of germination results to previously published data (Cavanagh, 1987b). A control was left untreated to provide baseline data on the non-dormant (not hard) proportion of each collection.

5. Dry Heat Shock

A full factorial of heat exposure at three temperatures (90°, 120° and 150°C) and four durations (1, 5, 10 and 30 min) was established. For all heat shock treatments involving *A. anthochaera* and *A. microbotrya* var. *microbotrya* (P1) a further four replicates of 25 seed were assigned for post-treatment tetrazolium tests. These tests aimed to determine whether the heat shock treatment reduced seed viability prior to incubation. The procedure used was as for the initial viability test.

6. Acid Scarification

Seeds from each collection were immersed in concentrated sulphuric acid (97%) under a fume hood for 10, 20, 40, 60, 80 and 100 min (and 120 min for both populations of *A. microbotrya* var. *microbotrya*). After acid treatment, seeds were rinsed thoroughly in distilled water prior to plating and incubation.

7. Data Analysis

Arcsine transformed percentage germination values were analysed by one-way analysis of variance and the Tukey-Kramer test using Statview ®.

Results and Discussion

The initial viability of all seed lots used for these experiments was high, with only the viability of *A. cyclops* (85%) and the two populations of *A. microbotrya* var. *microbotrya* (63% and 66%) being significantly less than 100% (Table 30.1).

The proportion of seeds exhibiting no dormancy, as defined by a control (no treatment) germination test, was generally low (Table 30.1) with *A. cyclops* having the highest proportion of non-dormant seed ($15 \pm 4\%$).

Germination after manual scarification did not differ from the mean viability determined using tetrazolium staining in four of the collections (*A. anthochaera* ($P = 0.15$), *A. cyclops* ($P = 0.15$), *A. lasiocalyx* ($P = 0.20$) and *A. microbotrya* ($P = 0.16$)) (Table 30.1). In all other cases germination was significantly less than that predicted from the tetrazolium tests and in the two *A. microbotrya* var. *microbotrya* collections, $< 50\%$ of those seeds predicted to be viable germinated. One possible reason for the lower than expected germination is that low vigour seeds, that nevertheless gave a positive tetrazolium result, succumbed to imbibition damage during germination tests. Imbibition injury has been reported in many species and is a particular problem in large seeded species in the *Fabaceae* (Ellis *et al.*, 1985). It is therefore likely that *Acacia* seeds would be susceptible.

The wet heat shock treatment improved the germination of *A. anthochaera*, *A. lasiocalyx*, *A. microbotrya* and *A. murrayana*. However, the resulting germination did not match the effectiveness of manual scarification. Germination after wet heat treatment did not differ from the control for the remaining seed lots (Table 30.1).

The results obtained in this study indicate that for the species and conditions investigated, dry heat shock was not an effective method for overcoming hard-seededness (data not shown). Only *A. lasiocalyx* showed any improvement in germination compared to the control. Maximum germination was still low with only 40% of seeds germinating, less than half the potential germination (99%) determined by manual scarification. Treatment at higher temperatures and longer durations caused a reduction in germination for most species examined. Elliot (2000) also confirmed that heat shock did not increase germination over a control for at least one of the study species (*A. microbotrya* var. *borealis*). The effectiveness of dry heat shock treatments for the alleviation of hard-seededness in *Acacia* appears to be species, site and seasonally specific. This is demonstrated by the variation in germination results presented for *A. cyclops* as reported by Jeffery *et al.* (1998), Jones (1963) and now the present study.

Table 30.1 Viability (%) determined by tetrazolium staining compared to germination (%) for control (no treatment), manual scarification and wet heat shock treatments for 8 *Acacia* collections. Species means indicated by * differ significantly from 100% viability ($P < 0.05$).

Species	Viability (% \pm se)	Control (% \pm se)	Manual Scarification (% \pm se)	Wet Heat Shock (% \pm se)
<i>A. anthochaera</i>	99 \pm 1	4 \pm 2	94 \pm 4	75 \pm 4
<i>A. cyclops</i>	85 \pm 2*	15 \pm 4	78 \pm 6	19 \pm 3
<i>A. lasiocalyx</i>	100	2 \pm 1	99 \pm 1	25 \pm 5
<i>A. microbotrya</i>	98 \pm 1	6 \pm 1	95 \pm 2	51 \pm 11
<i>A. microbotrya</i> var. <i>borealis</i>	100	3 \pm 2	89 \pm 1	6 \pm 2
<i>A. microbotrya</i> var. <i>microbotrya</i> (P1)	63 \pm 8*	1 \pm 1	30 \pm 3	1 \pm 1
<i>A. microbotrya</i> var. <i>microbotrya</i> (P3)	66 \pm 4*	5 \pm 3	10 \pm 3	4 \pm 0
<i>A. murrayana</i>	99 \pm 1	8 \pm 1	92 \pm 2	36 \pm 7

Acid scarification was found to be an effective method of breaking hard-seededness in all species investigated. The acid treatment time required to produce an improvement in germination ranged from 10 mins (*A. anthochaera*, *A. lasiocalyx* and *A. murrayana*) (Figures 30.1a, c & h) to 40 mins (*A. microbotrya* var. *microbotrya* (P3) and *A. microbotrya* var. *borealis*) (Figures 30.1e & g). Acid scarification was effective at reducing fungal infection of seed, possibly leading to increased germination (data not shown). The acid treatment time that gave the highest level of germination also varied between the species tested, possibly reflecting differences in seed coat thickness. However, with one exception, treatment times of 40, 60 and 80 min gave highest or near-highest germination, and in most cases the germination achieved was equal to that of the manual scarification treatment. The exception was *A. microbotrya* var. *microbotrya* (P3), which required 100 min (Figure 30.1g) and this treatment resulted in higher germination than that achieved with manual scarification. Other studies [for example, Rehman *et al.* (1999); Sacheti and Al-Rawahy (1998); Teketay (1998) and Cavanagh (1987b)] showed that an immersion time between 20 and 40 min was optimal for a range of *Acacia* species. In *A. lasiocalyx* and *A. microbotrya* var. *borealis*, highest germination following acid treatment was significantly less than that achieved in the manual scarification treatment (Figures 30.1c & e). It is possible that increased germination in *A. lasiocalyx*, *A. microbotrya* var. *borealis* and *A. microbotrya* could have been achieved given a longer treatment time. For these three species, hard seed remained at the conclusion of the experiment.

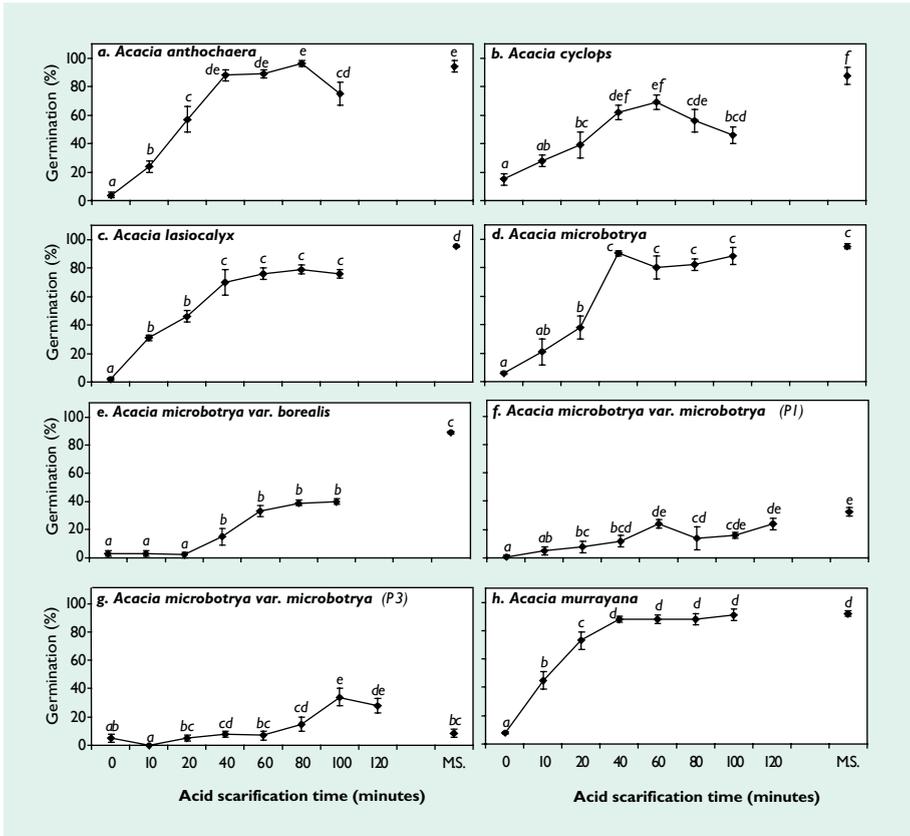


Figure 30.1 The effect of increasing period of exposure to concentrated sulphuric acid (97%) on final germination (%) after 14 d in eight *Acacia* collections. Germination after manual scarification (MS) is also shown. Treatment means found to be significantly different following the Tukey-Kramer test ($P < 0.05$) after arcsin transformation are represented by different letters.

This study has demonstrated that acid scarification is an effective method of overcoming hard-seededness in a range of *Acacia* species. This method can result in germination approaching the potential viability of the seed, as measured by tetrazolium staining and by germination following manual scarification and would be suitable for treating large quantities of *Acacia* seed. Acid immersion time to maximise germination can vary between species and between seedlots of the same species. The main disadvantages of the acid scarification technique are the risks associated with using such a corrosive chemical and the need for acid resistant equipment and personal protective apparatus for the user. Further investigation is required to determine whether it is possible to provide general guidelines for optimal immersion times for a range of species and provenances collected over different seasons.

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