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TECHNICAL PAPER NO. 39

**SEED GERMINATION TEST METHODS
USED FOR AUSTRALIAN TREE SPECIES
AT COFFS HARBOUR RESEARCH CENTRE**

BY

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FORESTRY COMMISSION OF NEW SOUTH WALES

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SEED GERMINATION TEST METHODS USED FOR
AUSTRALIAN TREE SPECIES AT COFFS HARBOUR RESEARCH CENTRE

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SUMMARY

This paper discusses the methods used for laboratory germination testing of Australian tree and shrub seed at the Forestry Commission of New South Wales, Coffs Harbour Research Centre. Details are given of sampling techniques, pre-treatments used to overcome seed dormancy, and equipment and methods used at each stage of germination testing. The types of records kept, calculation of results and assessment of seedlots are also discussed.

Tables are appended and list the results of germination tests and methods used for testing seed of 116 Australian tree and shrub species.

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1. INTRODUCTION

1.1 Overview

The object of seed germination testing is to determine the germinability and viability of seedlots and hence their suitability for sowing in nurseries or the field. In the Forestry Commission of New South Wales, test results are used to calculate sowing rates, to compare seedlots and to assess changes in seed viability during storage.

Rules for testing some tree seed germination were standardised by the International Seed Testing Association (ISTA Rules and Annexes, 1976, 1981 and 1985), but they did not include most Australian species.

Typical test methods used at Coffs Harbour until 1978 were described by Floyd (1964). Floyd's methods were based on those devised by Grose (1957) for *Eucalyptus* seed. Methods for other Australian species were adapted from Floyd's recommendations.

Some changes in Floyd's methods were considered necessary to bring them closer to ISTA testing procedures. The definition of germination was changed to that accepted by seed technologists (ISTA Rules, 1985). Seedlings are now evaluated at a later stage in development.

Appended to this paper are three Tables which list results of germination tests and methods used for testing seed of 116 species of Australian trees and shrubs. Most of the methods listed have not been fully researched and were used on only a small number of seedlots. References to authors who have more fully researched and recommended test methods for some of the species are included. *Eucalyptus* species are not included in the Tables as the test conditions recommended by ISTA (1985) and Boland *et al.* (1980) are used.

There is scope for more research on Australian tree and shrub seed. Possible areas of research include:

- Determining seed germination requirements, including pre-treatments needed to break dormancy
- Determining seed cleaning and storage requirements
- Studying seed viability and determining longevity in storage
- Setting standards for seed germination testing.

1.2 History of Forestry Commission of New South Wales Seed Store Management

Prior to 1978, the Commission's Amenity Seed Store was located at Pennant Hills nursery under ambient conditions. The North Coast *Eucalyptus* plantation seed was held in cold storage at Coffs Harbour. Germination testing was carried out on this seed at the Coffs Harbour Research Centre.

In 1978 the seed from Pennant Hills was moved into cold storage with the plantation seed at Coffs Harbour. The Amenity Seed Store

included seed of some 375 Australian tree and shrub species. There were approximately 200 *Eucalyptus* species, 60 *Acacia* species, 55 non-eucalypt Myrtaceous species of *Callistemon*, *Melaleuca*, *Leptospermum*, *Calothamnus*, *Lophostemon* and *Syncarpia*; as well as species of *Banksia*, *Allocasuarina*, *Casuarina*, *Callitris*, *Cassia*, *Brachychiton*, *Grevillea*, *Hakea*, and others. Most species were represented by only a few seedlots, often in small quantities. Not all species were tested. Seed was supplied to the Commission's Amenity Nurseries and also sold to the public and other organisations. Details of seed viability obtained from germination tests were given if requested, but ISTA certificates were not issued as Coff Harbour Research Centre was not an ISTA-accredited seed testing laboratory.

In 1985 the Commission ceased public sales and operating the Amenity Seed Store. Seed testing was limited to the species needed for the North Coast plantation nurseries.

2. DEFINITIONS

Seven important terms used in this manual are explained below.

2.1 Pure Seed

The ISTA Rules (1985) require that germination tests be carried out on pure seed and they define pure seed for many species. The Rules do not define pure seed for most of the Australian genera. However, the definition principles for pure seed given in the ISTA Rules and Annexes (1985) can be applied to many Australian species.

Problems arise when attempting to determine pure seed in some genera of the family Myrtaceae, e.g. *Eucalyptus*, *Callistemon*, *Melaleuca*, *Leptospermum*, *Lophostemon*, *Syncarpia*, *Calothamnus*. Seed of these genera is usually a mixture of seed and "chaff". Boland *et al.* (1980) defined *Eucalyptus* chaff as being "sterile particles derived from infertile and non-fertilised ovules".

Of all the Australian Myrtaceous genera, ISTA have only defined pure seed for *Eucalyptus* (ISTA, 1985). The ISTA definition of *Eucalyptus* pure seed is "Seed with or without testa. Piece of seed more than one-half the original size, with or without testa". This definition is not easily applied to many *Eucalyptus* species. The sterile particles may be similar in size and appearance to seed and are more numerous (Grose and Zimmer, 1958a). Separation of chaff from seed is therefore difficult. ISTA Rules (1985) state that "in many species of *Eucalyptus* it is impossible to differentiate with certainty between seed and ovulodes (= unfertilised or inhibited ovules that did not develop into mature seed)" and that "in some of these species it is extremely difficult to separate pure seed from inert matter composed of broken down fruit and inflorescence material". Similar problems occur with the other Myrtaceous genera mentioned above.

Germination tests are not carried out on equal numbers of pure seed of these genera, but using replicates of equal weight of the mixture of seed, chaff and inert matter.

2.2 Seedlot

A Forestry Commission of New South Wales seedlot is defined as a quantity of seed of a species contained, recorded and stored under one seedlot number.

2.3 Germination

Floyd (1964) used the criterion for germination as being the emergence and elongation of the radicle to one-sixth of an inch (about 4mm). Seed technologists measure germination at a later stage of development to attempt to assess the capacity of seed to give rise to healthy plants (Pollock and Roos, 1972). The seed technologist's definition of germination given in the ISTA Rules (1985) states that "germination of a seed in a laboratory test is the emergence and development of the seedling to a stage where the aspects of its essential structures indicates whether or not it is able to develop further into a satisfactory plant under favourable conditions in soil". The definition of germination given in the ISTA Rules (1985) is used at Coffs Harbour.

2.4 Normal Seedlings

During the germination test, seedlings are assessed for normal development. ISTA Rules (1985) define normal seedlings as those which "show the potential for continued development into satisfactory plants when grown in good quality soil under favourable conditions of moisture, temperature and light". They must be intact and have all their essential structures well developed, complete, in proportion and healthy. Some slight defects are acceptable if other structures are well-developed. The ISTA Annexes (1985) give detailed descriptions of normal seedlings and their essential structures.

2.5 Abnormal Seedlings

The ISTA Rules and Annexes (1985) describe abnormalities in three categories: damage, deformity and decay. Abnormal seedlings "do not show the potential to develop into a normal plant" under favourable environmental conditions.

Boland (1977) and Boland *et al.* (1980) discussed abnormalities of *Eucalyptus* seedlings. Published reports of abnormal seedlings in other Australian genera have not been found. Abnormalities which usually cause seedling death in *Eucalyptus* are poor radicle elongation, lack or paucity of root hairs, colourless and watery hypocotyl, dry and flaky hypocotyl, albino or chlorophyll deficient cotyledons, polyembryony (Boland *et al.*, 1980). Abnormal number of cotyledons does not always cause seedling death. However, such seedlings are classed as abnormal because of the uncertain effect of this condition.

Similar abnormalities are found to occur in other Myrtaceous species. For other genera, the ISTA Rules (1985) relating to abnormal seedlings (1985) are applied.

2.6 Germinability

A seedlot's germinability is the proportion of seeds in a sample which germinate and produce normal seedlings in a test.

2.7 Viability

A seedlot's viability is the proportion of living seed in the sample tested. Some of these seeds may be capable of germinating as soon as they receive adequate moisture and appropriate temperatures, but some may be dormant or hard (Peterson and Cook, 1981), and do not germinate in a test. Viable seed therefore includes germinable seed, plus ungerminated (dormant) and hard seed.

3. GENERAL TESTING PROCEDURE

In a germination test a sample of a seedlot is germinated under controlled environmental conditions in the laboratory. The procedure used for testing a seedlot is as follows:

1. A plan is made of the tests to be conducted.
2. The seedlots are sampled.
3. Each sample is reduced to replicates.
4. With seeds known to have some form of dormancy, the replicates are pre-treated with a treatment suitable for breaking the dormancy.
5. Replicates are given the appropriate temperature and light conditions for the germination phase of the test.
6. Seedlings are evaluated during the test period for normal development.
7. The test is completed.
8. Results are calculated and seedlots assessed for suitability for sowing, or re-tested if necessary.

4. PLANNING TESTS

A plan of tests is made several weeks in advance to ensure efficient use of limited staff, time and equipment. A pilot trial using a computer was successful in simplifying planning. The computer program searched Seed Store records and found those seedlots that required tests. These seedlots were then sorted on the basis of the environmental conditions required for the test. The factors that must be considered when planning are:

1. Time required for pre-treatment of seed
2. Temperature and light requirements for the species
3. Day of the week when the first evaluation will be made (day of first count)
4. Test duration.

Species having the same requirements are tested in batches of approximately ten tests. A consecutive number is allotted to each test.

It is desirable that new seed acquisitions be tested soon after they are put in store so that viability changes during storage can be assessed. Subsequent tests are conducted when viability figures are required for the Commission's plantation nurseries.

5. STORAGE AND SAMPLING

5.1 *Storage of Seed Lots*

Coffs Harbour has a warm, humid climate. Because these conditions are not ideal for seed storage, all seed is kept in sealed containers and stored in a refrigerated room maintained at 2-5°C. The relative humidity of this room is not controlled and is approximately 70%.

The seed handling area is adjacent to the Seed Store. Access to the seed handling area and Seed Store is through an air lock, which helps to isolate the Store further from the outside environment.

All seed is stored in sealed containers. Small seedlots (less than 5kg) are kept in glass or plastic screw-top jars. Seedlots over 5kg are stored in thick plastic bags inside tins or in sturdy plastic drums.

Moisture content of seed is only measured if the seedlot is to be used in plantation nurseries. The low constant temperature oven method described in the ISTA Rules (Annexe to Chapter 9, 1985) is used. If drying of the seed is necessary, it is put in a forced-air drying oven at 40°C until the seed moisture content drops to 10 - 12%. Paper packets of silica gel can be added to small seedlots to keep seed moisture content low.

No fungicides are used in storage of Australian tree and shrub seed. If insect activity is noticed in a seedlot, paper packets of naphthalene flakes are placed in the container of seed.

Little is known of the storage requirements and viability of Australian tree and shrub seed and this topic is outside the scope of this paper. It is generally accepted that constant low temperature and relative humidity is necessary for long-term storage of seed (Arvier, 1983). At Coffs Harbour, the high relative humidity of the seed storage room is partly compensated for by storage of seed in sealed containers at low temperature.

5.2 *Sampling Equipment and Methods*

It is only practicable to test the germination of a very small proportion of any seedlot and the results are meaningless if the sample tested is not representative of the seedlot (Justice, 1972).

The procedures and apparatus for sampling required in the ISTA Rules (Annexe to Chapter 2, 1985) are followed except where indicated below.

5.2.1 Sampling large seed lots. When sampling large seedlots over 5kg, small portions called primary samples are taken from different positions in the seedlot. A sleeve trier is used for this. The primary samples are combined to form a composite sample. The

composite sample is reduced to working sample size using a Gamet centrifugal divider. Instructions for using the trier and divider are given in the ISTA Rules (Annexe to Chapter 2, 1985).

The minimum working sample weights for some species are listed in the ISTA Rules (Annexe to Chapter 2, 1985). For species not listed, the minimum working sample size is 2500 seeds. For those species tested by weighed replicates, the minimum working sample size is ten times the recommended replicate weight. Replicate weights for 415 *Eucalyptus* species can be found in Boland *et al.* (Appendix 3, 1980). The Tables appended to this paper contain recommended replicate weights for other species tested by weighed replicates.

5.2.2 Sampling small seedlots. Seedlots less than 5kg are sampled by reducing the entire seedlot in the Gamet divider to a working sample size of approximately 500 seeds or five times the recommended replicate weight.

5.2.3 Hand sampling. Sampling of non-free flowing seeds is done by hand as directed in the ISTA Rules (Annexe to Chapter 2, 1985). The composite sample is reduced to working size by repeated mixing and dividing using the hand-halving method described in the ISTA Rules (Annexe to Chapter to 2, 1985) or the quartering method described by Grose and Zimmer (1958b). In the quartering method, the sample is placed onto a smooth surface, thoroughly mixed with a spatula, then quartered. Two opposite quarters are selected. Mixing and quartering are repeated until a working sample size of 500 seeds or five times the replicate weight is reached.

5.3 Sample Storage

Working samples are placed in an envelope labelled with the species name and seedlot number.

It is desirable to keep the time between sampling and testing to a minimum. If possible, tests are begun the same day as samples are taken. Justice (1972) recommended that seed samples awaiting tests be stored under conditions that will maintain their original quality. If samples must be stored, they should be sealed and placed in a refrigerator.

6. THE GERMINATION TEST

The ultimate object of making a germination test is to gain information about the field planting value of the seed and to provide results which can be used to compare the value of different seedlots (ISTA Rules, 1985).

The test conditions must supply adequate moisture, a suitable temperature, and a suitable growing medium or substrate for the seed (Mackay, 1972). ISTA aims to develop methods which control some or all external conditions to give "the most regular, rapid and complete germination for the majority of samples of a particular species" (ISTA, 1985). It is important to remember the object of germination testing when developing test methods that maximise germination. The evaluation of normal and abnormal seedlings is an attempt to move the germination test results closer to the field planting value of seedlots (Justice, 1972).

The germination test conditions used for testing Australian species are described below.

6.1 Substrates

Most seeds are germinated in 10 cm or 12 cm glass petri dishes using substrates of filter paper and vermiculite (Larsen, 1965). Glass dishes are easily labelled, cleaned and sterilised.

Germinating small seeds on top of filter paper allows them to be easily seen and does not hinder root development of small seedlings. Vermiculite is usually used as a porous base under filter paper to provide adequate moisture during the test.

Larger seeds can be germinated on vermiculite alone, so that root development is not hindered and seeds are in better contact with water.

Larger trays are used for seeds such as some banksias, to allow sufficient space for seedling development. The trays used are commercial meat trays that have a clear plastic lid and dimensions of 30 cm x 20 cm x 4 cm (manufactured by Aladdin).

The substrates are set up as follows:-

6.1.1 TPV: Top of paper and vermiculite. This is the most commonly used method. A measured quantity of agricultural grade vermiculite is put in the base of the petri dish. The vermiculite is levelled and moistened with water. A piece of Whatman No. 1 filter paper is then placed on top of the moist vermiculite. Table 1. lists the quantities of vermiculite and water used for the two sizes of petri dish.

6.1.2 TP: Top of Paper. Five pieces of Whatman No. 1 filter paper are placed in the base of a petri dish and moistened with water (for quantities, see Table 1). Petri dishes that seal well are used because this method is only suitable for species with small seeds that are not likely to dry excessively during the test, e.g. most Myrtaceous species that have seed/chaff mixtures.

6.1.3 TV: Top of Vermiculite. A measured quantity of vermiculite is put in the base of the petri dish. It is labelled and moistened with water (for quantities, see Table 1). If a tray is used for a large-seeded species, a layer of vermiculite about 5 mm deep is put in the bottom of the tray and moistened until damp and all free water has been absorbed by the vermiculite.

Some species of *Eucalyptus* contain inhibitors in the chaff or seeds that leach out during germination and interfere with normal seedling development (Boland *et al.*, 1980). A substrate of vermiculite alone allows these leachates to drain away from direct contact with seedlings. However, only the larger-seeded species of *Eucalyptus* are tested using this method e.g. *E. citriodora*, *E. maculata*. Smaller seeds are not easy to see on top of vermiculite only. They may fall between the grains of vermiculite and so stay in contact with the leachates. These seeds are tested using the TPW method.

Table 1. Quantities of filter paper, vermiculite, and water used for TPV*, TP, and TV substrates

Germination	TPV		TP		TV	
Substrate						
Petri dish size (cm)	10	12	10	12	10	12
Filter paper:						
Number of pieces x diameter (cm)	1 x 9	1 x 11	5 x 9	5 x 11	-	-
Vermiculite (g)	4	8	-	-	4	8
Water (ml)	25	50	5	10	20	40

* TPV = top of paper and vermiculite; TP = top of paper; TV = top of vermiculite

6.1.4 *TPW: Top of Paper and Watchglass*. This method is adapted from Grose and Zimmer (1958b) and is illustrated in Figure 1. A 10 cm petri dish is inverted and a cotton wick placed around the inside perimeter of the base. Two pieces of 11 cm Whatman No. 1 filter paper are cut radially (Fig. 1b) and placed on the convex face of a 9 cm watchglass. The paper is moistened and smoothed and the edges folded under the watchglass. The watchglass is placed convex side up in the dish so that its perimeter rests on the wick (Fig. 1a). 15 mL of water is added to the reservoir around the wick.

The TPW method was used for most tests before 1978. It is rarely used now as it is more time consuming to set up and evaporation is greater than with the preferable TPV method. However, it is useful for testing the smaller *Eucalyptus* seeds which have inhibitory leachates, e.g. *E. calycogona*, *E. cloeziana*, *E. haemastoma*, *E. intertexta*, *E. kruseana*, *E. melliadora*, *E. microtheca*, *E. resinifera*. On the paper covered watchglass, the seeds are easily seen and leachates can drain away.

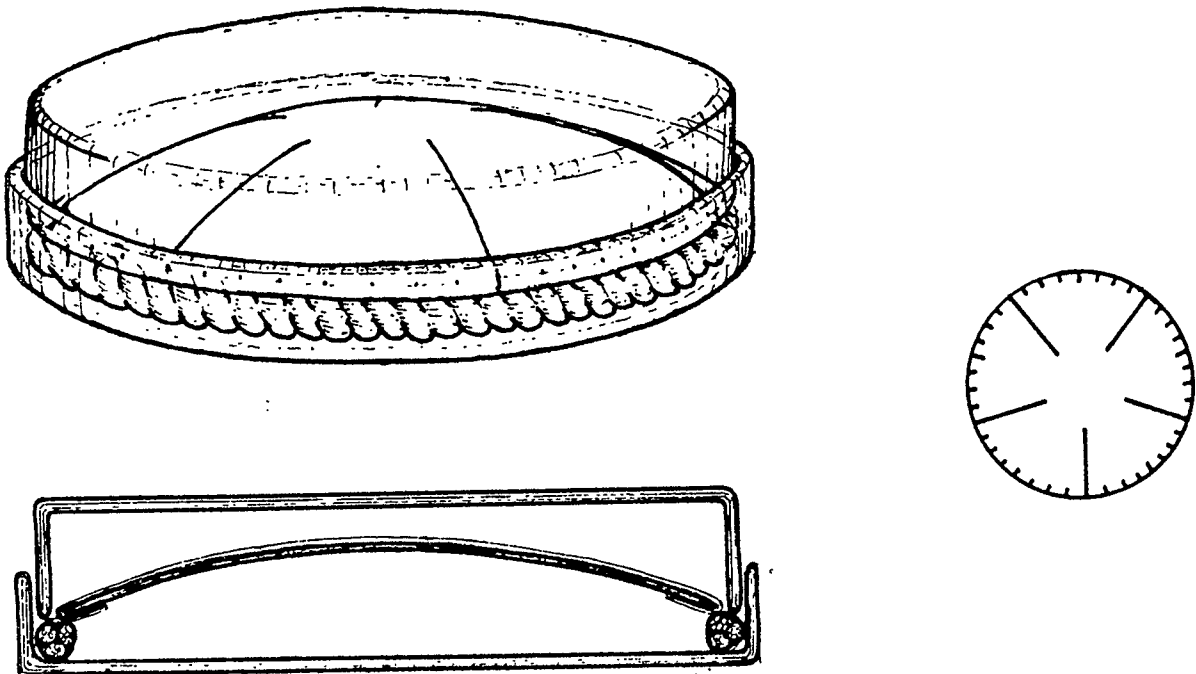


Figure 1a. Petri dish with filter paper over watchglass and wick, and section through dish.

Figure 1b. Radial cuts on filter paper

Figure 1. TPW Substrate

6.2 Replicates

Once the appropriate substrates have been prepared, the working sample is reduced to replicates.

6.2.1 *Replicates by seed number*. For species tested using replicates of equal number of seeds, 400 seeds are taken at random from the

working sample. They are divided into replicates of 4 x 100 seeds, 8 x 50 seeds or 16 x 25 seeds, depending on seed size.

Each replicate is weighed to the nearest mg and the average weight per replicate is calculated.

6.2.2 *Replicates by seed weight.* Seedlots of Myrtaceous species that have seed/chaff mixtures are tested using replicates of equal weight (see Section 2.1). The aim is to test a weight of seed mixture containing approximately 100 seeds per replicate. The recommended weights in the appended Tables have been calculated from data obtained from germination tests of each species. This data includes the average number of germinable, ungerminated, abnormal and dead seeds, but does not include "empty" seeds, for reasons discussed in Section 2.1.

Four replicates of the recommended weight (to the nearest mg) are taken from the working sample.

6.3 *Seed Pre-treatments*

Seed of some species tested will not germinate when exposed to conditions favourable for germination without some type of pre-treatment. These seeds are dormant. Villiers (1972) described dormancy as being the state of arrested development whereby seeds possess some mechanism preventing their germination.

Villiers (1975) classified seed dormancy into two types:

- (a) Dormancy imposed by the seed coat, e.g. hard seed of the Leguminosae. This type of dormancy is broken by treatments which scratch or crack the seed coat, allowing water and gases to enter.
- (b) Embryo dormancy, which is due to a metabolic block within the embryo itself. This can be broken by conditions which bring about physiological changes in the embryo, such as light, chilling or dry storage.

Some eucalypts require pre-chilling or light to germinate, but it has not yet been determined what type of dormancy these have (Boland *et al.*, 1980).

The test results will be more representative of the nursery situation if similar pre-treatments are used for the test as in the nursery. Some pre-treatments that give maximal germination in a test are not practical for use in the nursery.

The methods listed below (Sections 6.3.1 to 6.3.5) are used to break and induce germination in a test. Each treatment has a code letter which is also used in the Pre-treatment column of Tables 1, 2 and 3 in the Appendix.

After seed has been pre-treated for a test, each seed replicate is placed on the moist substrate and the seeds uniformly spaced. The lid of the petri dish is labelled with test and replicate numbers and placed over the seeds.

6.3.1 *Acid Scarification (A)*. This is one of two methods recommended by ISTA (1985) for treatment of hard seeds of acacias. ISTA (Amendments, 1981) recommends the other method, hand scarification, as being more reliable. There is evidence that acid is not an effective pre-treatment for acacias (*pers. comm.*, D. Boland, Tree Seed Centre, Division of Forest Research, C.S.I.R.O., Canberra). However, it may be useful for species not yet tested.

Each replicate, after weighing, is put in a separate test tube or beaker. Concentrated sulphuric acid is then carefully poured over the seed to cover it. The seed is then left to soak until the testa is pitted. Seeds are examined every 10 minutes until this occurs. The acid is then drained off and the seed put in a wire mesh strainer (each replicate being kept separate) and rinsed thoroughly under running cold water. Care must be taken at all stages during the pre-treatment because of the danger of chemical burns to the operator. A problem with soaking in acid is that seeds may be unevenly treated and some could be damaged.

6.3.2 *Hand Scarification (S)*. This term covers several techniques, each of which can be used to treat hard seeds. They are either filed with a flat-blade file, pierced with a needle or the testa is chipped with a scalpel.

After weighing the replicates, a fragment of the testa of each seed is chipped off or filed, or the seed is pierced, at the cotyledon end. The ISTA Rules (Annexe to Chapter 5, 1985) note that the best site for scarification, to avoid damaging the embryo and resulting seedling, is that part of the seed coat immediately above the tips of the cotyledons. Piercing usually gives the highest germination with fewer abnormalities than chipping or filing (ISTA Rules, Amendments, 1981).

A quicker method of hand scarification is to rub each replicate between two pieces of sandpaper. However, this may not evenly treat all seeds.

Seeds are soaked after hand scarification, usually for three hours, in cold water.

6.3.3 *Hot water (HW); Boiling water (BW)*. This method is also used to treat hard seeds. The high water temperature causes the seed coat to crack, allowing the seeds to imbibe water. After weighing, each replicate is put in a beaker and 100 mL of hot water (80°C) or boiling water as recommended, is poured over the seed. The seed is allowed to soak in the cooling water for three hours. It is then rinsed thoroughly in cold water as some species exude mucilaginous material when soaked.

It should be noted that scarification treatments are probably more reliable and may allow higher and more rapid germination of a seed sample than does treatment with hot or boiling water. But this is not always the case. Clemens *et al.* (1977) found that two species of *Acacia* gave lower germination responses with scarification than with hot water treatments. The disadvantage of the hot or boiling water treatment is that it may not affect all seeds in the sample. Careful hand scarification should allow entry of water/gases into all the seeds treated. Seeds subjected to the water treatment may require

different temperatures and length of treatment. Clemens *et al.* (1977) found differing responses to hot water temperature and length of treatment in five *Acacia* species. Germination increased with severity of treatment up to a point where seed mortality was reached. Buszewicz (1978) reported that length of optimum treatment varied with species and seedlot when the boiling water treatment was used. The ISTA Rules (Annexe to Chapter 5, 1985) recommend hand or acid scarification for acacia seed but also state that hardseededness in acacias may be treated by plunging the seeds in about three times their volume of nearly boiling water and leaving them to soak until the water cools.

In Forestry nurseries, it is impractical to hand scarify hard seed. The simpler hot or boiling water treatment is used, and the seeds are left to soak up to 24 hours in the cooling water before sowing. Therefore, when testing acacias, it is considered appropriate to use the hot or boiling water treatment, because the test results are used to calculate nursery sowing rates.

6.3.4 Pre-chilling (PC). Pre-chilling, also known as cold-moist stratification, can be used to break embryo dormancy in some eucalypts (Boland *et al.*, 1980). This treatment subjects seeds to a cold moist environment. Seed replicates are weighed and put on the moist substrate. The lids of the dishes are labelled with test and replicate numbers and are placed over the seeds. Before being placed in the germination cabinet, the replicates are put in a refrigerator (5°C) for the recommended time.

The replicates are regularly checked for moisture as seeds imbibe water during stratification. Water is added as needed. When the pre-chilling period is over, the replicates are removed from the refrigerator to the germination cabinet.

6.3.5 Cold Water Soak (CW). Seeds of some species such as *Atriplex* contain chemical inhibitors which must be leached out before germination can occur. Beadle (1952) recommended soaking the fruits in water for 24 hours, provided all excess water was subsequently removed from the fruits to prevent waterlogging the seed. This was done by squeezing the fruits between pieces of blotting paper.

Other species which may respond to cold water soaking are some species of *Grevillea* and *Hakea*. For these species, each replicate is weighed and soaked in 100 mL of cold water for 24 hours or longer when indicated. The water is then drained off.

6.4 Temperature, Light and Humidity

Seed is germinated in a cabinet which provides controlled environmental conditions of temperature, light and humidity. The internal dimensions of the cabinet are 54 cm x 102 cm x 58 cm.

Temperature can be adjusted from 0-50°C, and also alternated to simulate low night/high day temperatures. The only alternating temperature cycle used is 20°C for 16 hours, followed by 30°C for 8 hours (20/30°C).

Light is provided by a bank of cool white fluorescent lights at the back of the cabinet. A daily cycle of 8 hours light and 16 hours dark

is used unless a special light regime is required. Lights can be set to coincide with the high day temperatures on an alternating temperature cycle.

Humidity in the cabinet is maintained automatically at about 90%.

The cabinet was specially constructed and is annually serviced by the New South Wales Department of Public Works Electrical Services Workshop, Glebe, New South Wales.

The cabinet controls are set to provide the recommended conditions at least one hour before the commencement of a test to allow time for the temperature and humidity to reach the required level.

When the replicates have been prepared and if necessary pre-treated, they are put in the germination cabinet.

6.5 Testing New Species

Many species in the Seed Store were represented by only one or two seedlots. It was therefore difficult to determine the optimum conditions required for germination of the species.

Time usually does not permit testing of a new species over the full temperature range in the germination cabinet. At this Research Centre there is no thermo-gradient apparatus (Fox and Thompson, 1971) which allows a species to be tested over a range of temperatures from 0-50°C at the one time.

Therefore, when a new species is to be tested, the first test is conducted at 25°C or 20/30°C. Lights are set for 8 hours. If the alternating temperature cycle 20/30°C is used, lights are set to coincide with the 8 hour 30°C part of the cycle. The TPV substrate is used if the species has small seeds, TV if the seeds are larger and easily visible on vermiculite, e.g. *Acacia*, *Albizia*.

If germination is satisfactory using these conditions, subsequent tests are carried out using the same. If germination appears to be slow or irregular, other temperatures are then tested.

When dormancy is suspected, a test is made using the pre-treatment considered appropriate and an untreated test used as a control. These germination tests are run concurrently.

When testing a new species which requires weighed replicates, the first test is carried out using 0.10 g seed per replicate. If the number of seeds is significantly less than 100 per replicate, the seed lot is re-tested using a larger weight per replicate.

6.6 Moisture

De-ionised water is used for testing seed. During the test, water is added to the substrate as needed. A syringe is used to trickle small amounts of water down the side of the petri dish so there is minimal disturbance to seeds and seedlings.

ISTA Rules (Annexe to Chapter 5, 1985) require that at all times the substrate must contain sufficient but not excessive moisture for

germination. ISTA Rules (1976) recommend for tests on paper, that the paper should not be so wet that when pressed, a film of water forms around the finger. This "film of water" test was investigated by Peterson and Cooper (1979) who found it unsuitable and suggested that moisture potentials be pre-determined for each species. They also found great variation in individual analysts' interpretations of the amount of water needed in a test. However, the film of water test is the present method used for estimating water needs of a test. It is considered better to err on the side of too little moisture than too much. For tests on vermiculite, water is added until the substrate is moist and all free water is absorbed by the vermiculite.

6.7 Germination Test Records

For each test, a germination test sheet is prepared to record details of the species and seedlot under test, the test number, the pre-treatment, temperature, substrate and light conditions used and the date of test commencement. Germination and test results are recorded on this sheet (see Fig. 2a and 2b).

Comments are recorded concerning unusual features of the test or germination, such as types of abnormalities, poor seedling growth, delayed germination, replicates drying out, etc.

6.8 Seedling Evaluation

The day of commencement of the test is treated as Day 0. The first count of germinated seedlings is made on the day recommended for each species concerned. Occasionally it is made a few days earlier or later to allow for accurate evaluation of seedlings. Further counts are made every few days at the analyst's discretion. At each count seedlings which are sufficiently developed are evaluated as normal or abnormal according to the definitions in Section 2.4 and 2.5. Once classed, normal seedlings are removed with forceps from the dish, and the number per replicate recorded.

Abnormal seedlings which are not decayed are usually left on the substrate until the final count. Seeds or seedlings which die and decay during the test are removed so they are not a source of contamination to healthy seedlings. At each count the number of dead seeds and abnormal seedlings removed per replicate is recorded on the germination test sheet, in the appropriate box at the lower part of the table.

6.9 Test Duration and Completion

The duration of the test period does not include the period of time taken to pre-treat the seed. The recommended duration of the germination test for a species is called the "day of final count". It is preferable not to extend tests beyond one week of the recommended day of final count, except for hardseeded species (see Section 6.9.2 (d)). Extension of the test may give higher germination but these slower germinating seedlings are often poor or abnormal. Verhey (1960) noted that seedlings kept for too long in an environment of high relative humidity can become weak and watery and that, in such an environment, fungi may be too virulent and seedlings too susceptible to infection.

On the day of final count after any germinated seedlings have been classed and removed, any remaining seeds are evaluated by cutting or squashing. Soft seeds are squashed with a spatula, harder seeds are cut with a scalpel. These seeds are classed according to the definitions given below (and in the ISTA Rules, 1985), as being either hard, fresh ungerminated (presumed dormant), empty or dead.

6.9.1 Hard seeds. Seed of some legumes may at the end of the test remain hard. Although pre-treated, some of these seeds may not have imbibed water, indicating that the pre-treatment was not effective on these seeds. If the number of hard seeds is greater than 25%, the seedlot is re-tested, except in the case of *Acacia* species (see Section 7.3).

6.9.2 Ungerminated seeds. Seeds, other than hard seeds, that remain firm, and apparently viable but have not germinated by the end of the test period are classed as ungerminated ("fresh ungerminated", ISTA, 1985). Included in this class are seeds which are late germinating but the seedlings have not yet reached the stage of development at which they can be readily evaluated as normal or abnormal. If it is apparent, without squashing or cutting the seeds, that more than 20% of seeds are still ungerminated (not obviously dead) at the end of the test period, the test is extended one week. Delayed germination can be caused by several factors:

- (a) Some form of dormancy may be present. This is only suspected in a species which has not been previously tested. A re-test may be necessary using an appropriate pre-treatment (see Section 7.3).
- (b) Deterioration of the seedlot. Often many abnormal seedlings or dead seeds are also present (see Section 7.4).
- (c) Small differences in germination conditions required e.g. the particular seedlot may not respond to the recommended temperature which is suitable for most seedlots of that species. This may occur with a seedlot from a different provenance not previously tested. A re-test can be done using a different temperature.
- (d) Some hardseeded species germinate over long periods, even after pre-treatment. This sometimes occurs when the hot or boiling water treatment is used. If germination of these species is still occurring after the recommended test period has elapsed, the test is extended up to three weeks.

6.9.3 *Empty seeds*. Seeds which are less than half-filled with live, dead or decayed embryonic and endosperm tissue at the end of the test are classed as "empty seeds". The empty seeds class is not applied to those species of the Myrtaceae family which are tested by weighed replicates. In these species, there are problems in differentiating between empty seeds and chaff (see Section 2.1).

6.9.4 *Dead seeds*. Seeds which at the end of the test period are not hard, ungerminated or empty are classed as 'dead seeds'. When squashed or cut, these seeds are usually decayed and discoloured.

The number of dead seeds removed during the test is added to the number of dead seeds squashed at the end of the test when calculating results.

6.10 *Hygiene*

It is important that a high standard of hygiene is maintained in germination testing. After tests are completed the dishes are washed thoroughly. Glass petri dishes are steam sterilised for 10-15 minutes. Plastic trays are soaked in 0.0125% Milton solution (active ingredients sodium hypochlorite 1% and sodium chloride 16.5%) for a minimum of one hour. The inside of the germination cabinets are cleaned once per month with this solution.

7. RESULTS AND ASSESSMENT

7.1 *Calculation of Results*

The number of normal seedlings that germinate in a test is expressed as a percentage of the total number of seeds tested. This figure is called "germination per cent". The number of abnormal seedlings, ungerminated seeds, dead seeds, empty seeds and hard seeds where applicable, are also each expressed as a percentage. Where replicates of seed weight are tested, the total number of seeds is variable but germination is still expressed as a percentage of the number of seeds tested, excluding empty seeds (see Section 6.9.3).

When more than four replicates are used (i.e. 8 x 50 seeds or 16 x 25 seeds), results for 4 x 100 seed replicates are formed by combining results from those replicates which were closest together in the germination cabinet (Miles, 1963).

A calculation is made of the number of germinable seeds per unit weight of seed. This figure is based on the mean weight of the replicates and the mean number of normal germinations per replicate, and is used for determining sowing rates. The number of viable seeds per unit weight, which includes ungerminated seeds and normal germinations, and hard seeds where applicable, is also calculated. This figure is used to determine sowing rates for hardseeded species which may germinate over long periods. Tables 1, 2 and 3 of the Appendix list viability figures for the species tested. Figures 2a and 2b are examples of the germination test sheets used, and show the calculation of results.

An index of germination rate called the "Germination Energy Index" (G.E.I.) (Grose and Zimmer, 1958a and 1958b) was used for some *Eucalyptus* seed lots (Floyd, 1964). The concept of "energy" for

measuring the speed of germination was based on the idea that the quicker seeds germinate, the better their quality (Verhey, 1960). Delay in the full expression of germination is usually the earliest detectable sign of quality loss in a seed lot (Abdul-Baki and Anderson, 1972). Seed which germinates quickly in the nursery provides an obvious advantage. However, the G.E.I. is no longer used because it cannot be accurately calculated when germination is measured at the later stage of seedling development. Pollock and Roos (1972) noted that there was no point in seedling development beyond radicle emergence which could be precisely timed. Tests at this Research Centre have shown that when germination was counted at later stages in seedling development, G.E.I. was not a useful test index as it did not sufficiently reflect the nursery performance of seedlots.

The Kilogram Effective Factor (KEF) described by Carter (1979) and Seward (1980), may be a more practical alternative index to the G.E.I. for use in plantation nurseries. The KEF index predicts the nursery yield of plantable seedlings from sowing a known quantity of a given seedlot (Carter, 1979). The usefulness of KEF has not been evaluated at Coffs Harbour.

7.2 Tolerances

Some variation is generally found in germination test results between replicates in a test and between tests of the same seedlot. There are many reasons for this variation, including: chance, poor method, poor technique, inconsistency in distinguishing between normal and abnormal seedlings, fungi or bacteria, chemicals on the seed, inaccurate counting or recording, non-random sampling, actual change in percent germination between tests (Miles, 1963).

Tolerance tables have been published by ISTA (Miles, 1963; ISTA Rules and Annexes, 1985) which list the maximum acceptable variation (called tolerance or tolerated range) between results. These tables are used for percent germination, percent ungerminated seeds, percent dead seeds, percent abnormal seedlings, percent hard seeds or the sum of any of these attributes.

There are different tables for different situations and the main tables used are Miles (Table G1, 1963) for comparing replicates within tests, and Miles (Table G2, 1963) for comparing tests of the same seedlot.

The average percent of the four replicates is calculated (combining 8 x 50 or 16 x 25 seed replicates to form 4 x 100 seed replicates as described in Section 7.1) and the corresponding maximum tolerated range is read from the table. When the results are outside the tolerated range, they are significantly different, and the seedlot is re-tested. However, if one replicate is out of tolerance for obvious reasons (e.g. drying out, fungal infection) the results of that replicate are omitted and the percent germination calculated on the remaining three replicates.

Tolerance tables for tests using weighed replicates were published by ISTA (Rules and Annexes, Appendix C, 1985).

7.3 Re-Testing

As discussed in Sections 6.9.1. and 6.9.2 (a) when more than 25% hard seeds or 20% ungerminated seeds remain at the end of the test and dormancy is suspected, the seedlot is re-tested using a different temperature or a pre-treatment considered appropriate. The exception is for *Acacia* species which were tested using the HW or BW pre-treatment. These are not re-tested using other pre-treatments (see Section 6.2.2). If the number of dead seeds is abnormal

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APPENDIX

The following Tables list germination test methods used or tried at Coffs Harbour since 1975, and the results obtained from the tests. Table 1 lists the results of germination tests and the methods used for 97 species. This data is taken only from tests which resulted in over 50% viability. Table 2 lists results and germination test methods for 9 species which may have a large proportion of empty seed. Table 3 lists results and germination test methods considered to be unreliable, for 10 species that have not been successfully tested using the methods listed.

The methods listed in the Tables have not been fully researched and were used on a small number of seedlots of each species. The Tables include references to authors who have recommended test methods for some of the species tested. These methods are used if they have been well researched. Although most of the tests at Coffs Harbour are on *Eucalyptus* species, none are listed in the Tables. The ISTA Rules (Annexe to Chapter 5, 1985) list test conditions (temperature, substrate, replicate weight and test duration) for 47 *Eucalyptus* species. Boland *et al.* (Appendix 3, 1980) recommend test conditions for 415 *Eucalyptus* species. These test conditions are used for the *Eucalyptus* species tested at Coffs Harbour.

An explanation of the column headings in the Tables follows:

Number of seeds/g - range. Lists for each species, the range of number of seeds per gram found for the seed lots tested for the Coffs Harbour Seed Store. The letter "a" appears in this column for the Myrtaceous species. As discussed in Sections 2.1 and 6.9.3, it is difficult to differentiate empty seed from chaff in many of these species, and the proportion of viable seed to chaff may vary greatly.

Number of germinable seeds/g - mean. Lists for each species, the average number of seeds which germinated per gram, in the seedlots tested (see Section 2.6).

Number of viable seeds/g - mean. Lists for each species, the average number of viable seeds per gram in the seedlots tested (i.e. germinable seeds plus ungerminated seeds, plus hard seeds where applicable - see Section 2.7).

Number of viable seeds/g - highest recorded. Lists the highest number of viable seeds per gram recorded for the species. Where only one seedlot of a species has been tested, only the figure for the mean number of viable seeds per gram is given.

Replicates (see Section 6.2). Replicates are either by number of seeds i.e. 4 x 100 seeds, 8 x 50 seeds or 16 x 25 seeds, or by a weighed quantity of seed (grams). For species tested by weight, the recommended replicate weight has been calculated from the average number of germinable seeds plus ungerminated, abnormal and dead seeds for the seedlots tested, to give approximately 100 of these seeds/replicate.

Temperature (see Section 6.4). Temperatures listed (in °C) are those that were found satisfactory. A full range of temperatures was not tested for any of the species listed, except for some tested by other

authors as indicated. Most seed has only been tested at 25°C. Where two temperatures are listed, both have been found satisfactory. Light is used for 8 hours per day for all species.

Substrates (see Section 6.1). The abbreviations used are:

- TPV - Top of Paper and Vermiculite
- TP - Top of Paper
- TV - Top of Vermiculite

Where two substrates are listed, both have been found satisfactory. Where "12cm dishes" or "trays" is noted, the seed of the species were too large to be germinated in 10cm petri dishes and the larger dishes or plastic trays were used.

Pre-Treatment (see Section 6.3). Abbreviations used are:

- A - Acid scarification
- S - Hand scarification
- HW - Hot water (100mL, 80°C), soak for three hours in the cooling water.
- BW - Boiling water (100mL), soak for three hours in the cooling water.
- CW - Cold water (100mL at ambient temperature), soak for 24 hours, unless indicated otherwise.

Day of first count (see Section 6.8). Day on which the first count of germinated seedlings is recommended.

Day of final count (see Section 6.9). Day on which the final count of germinated seedlings is recommended.

Number of seedlots tested; number of tests. This is the number of seed lots of that species tested since 1975; followed by the total number of tests carried out on the species. In some cases, the results of more than one test on a seedlot have been included.

TABLE 1. RESULTS OF GERMINATION TESTS AND METHODS USED

SPECIES	NUMBER OF SEEDS/g RANGE	NUMBER OF GERMINABLE SEEDS/g MEAN	NUMBER OF VIABLE SEEDS/g MEAN	NUMBER OF VIABLE SEEDS/g HIGHEST RECORDED	REPLICATE X NUMBER SEEDS OF WEIGHT (g)	TEMPERATURE (°C)	SUBSTRATE	PRE-TREATMENT	DAY OF FIRST COUNT	DAY OF FINAL COUNT	NUMBER OF SEED LOTS TESTED; NUMBER OF TESTS	COMMENTS
Acacia spp.					4 x 100 8 x 50 or 16 x 25	25† 20;20/30†	TV 12cm dishes	BW† S†	7	35† 21†	1:1	†Used at Coffe Harbour. †ISTA (1985) recommends 20 or 20/30°C temperature. A or S pre-treatment and 21 days. ISTA (1981) recommends S treatment followed by 3 hour soak in cold water as being the most reliable.
Acacia acuminata	39†	3†	20†		4 x 100	25	TV 12cm dishes	BW	7	35	1:1	†Wet seed weight.
Acacia adunca	20	3	15		8 x 50	25	TV 12cm dishes	BW	7	35	1:1	
Acacia bailevana	35-51	17	35	45	4 x 100	25	TV 12cm dishes	BW	7	35	3:3	
Acacia concurrens	99	72	79		4 x 100	25	TV 12cm dishes	BW	7	35	1:1	
Acacia conferta	54-111	18	81	111	4 x 100	25	TV 12cm dishes	BW	7	35	2:2	
Acacia cultriformis	59	10	30		4 x 100	25	TV 12cm dishes	BW	7	35	1:1	
Acacia dealbata	79	10	69	70	4 x 100	25	TV 12cm dishes	BW	7	35	1:2	
Acacia deanei	36	3	18		4 x 100	25	TV 12cm dishes	BW	7	35	1:1	
Acacia decora	57†	27†	54†		4 x 100	25	TV 12cm dishes	BW	7	35	1:1	†Wet seed weight
Acacia decurrens	66	1	61		4 x 100	25	TV 12cm dishes	BW	7	35	1:1	
Acacia elata	22-26	15	19	20	8 x 50	25	TV 12cm dishes	BW	7	35	2:2	
Acacia falcata	72-74	1	73	73	4 x 100	25	TV 12cm dishes	BW	7	35	2:2	
Acacia farnesiana	9†	1†	9†	9†	8 x 50	25	TV 12cm dishes	BW	7	35	2:2	†Wet seed weight.

SPECIES	NUMBER OF SEEDS/G RANGE	NUMBER OF GERMINABLE SEEDS/G MEAN	NUMBER OF VIABLE SEEDS/G MEAN	NUMBER OF VIABLE SEEDS/G HIGHEST RECORDED	REPLICATE X NUMBER SEEDS OF WEIGHT (g)	TEMPERATURE (°C)	SUBSTRATE	PRE-TREATMENT	DAY OF FIRST COUNT	DAY OF FINAL COUNT	NUMBER OF SEED LOTS TESTED; NUMBER OF TESTS	COMMENTS
<i>Acacia flemingii</i>	67-104	27	75	86	4 x 100	25	TV 12cm dishes	BW	7	35	2:2	
<i>Acacia gladiiformis</i>	43	14	31		4 x 100	25	TV 12cm dishes	BW	7	35	1:1	
<i>Acacia homalophylla</i>	94-113	9	51	52	4 x 100	25	TV 12cm dishes	BW*	7	35	1:2	*Pre-treatment used in tests not recorded, recommend BW
<i>Acacia implexa</i>	47	3	46		4 x 100	25	TV 12cm dishes	BW	7	35	1:1	
<i>Acacia intertexta</i>	79	50	57		4 x 100	25	TV 12cm dishes	BW*	7	35	1:1	*Pre-treatment used in test not recorded, recommend BW
<i>Acacia iteaphylla</i>	28	1	26		8 x 50	25	TV 12 cm dishes	BW	7	35	1:1	
<i>Acacia leiocalyx</i>	101	65	83		4 x 100	25	TV 12 cm dishes	BW	7	35	1:1	
<i>Acacia ligulata</i>	21*	3*	19*		8 x 50	25	TV 12 cm dishes	BW	7	35	1:1	*Net seed weight
<i>Acacia linearifolia</i>	69	36	64		4 x 100	25	TV 12 cm dishes	BW	7	35	1:1	
<i>Acacia linifolia</i>	29	27	27		8 x 50	25	TV 12 cm dishes	BW*	7	35	1:1	*Pre-treatment used in test not recorded, recommend BW
<i>Acacia linophylla</i>	13	8	9		8 x 50	25	TV 12cm dishes	BW*	7	35	1:1	*Pre-treatment used in test not recorded, recommend BW
<i>Acacia longifolia</i> var. <i>longifolia</i>	95	41	67		4 x 100	25	TV 12cm dishes	BW	7	35	1:1	
<i>Acacia longifolia</i> var. <i>sophorae</i>	39-57	17	27	28	4 x 100	25	TV 12cm dishes	BW	7	35	2:2	
<i>Acacia melanoxylon</i>	81	58	80		4 x 100	25	TV 12cm dishes	BW	7	35	1:1	
<i>Acacia microcarpa</i>	193	4	143		4 x 100	25	TV 12cm dishes	BW	7	35	1:1	
<i>Acacia oswaldii</i>	11	3	6		8 x 50	25	TV 12cm dishes	BW*	7	35	1:1	*Pre-treatment used in test not recorded, recommend BW

SPECIES	NUMBER OF SEEDS/G RANGE	NUMBER OF GERMINABLE SEEDS/G MEAN	NUMBER OF VIABLE SEEDS/G MEAN	NUMBER OF VIABLE SEEDS/G HIGHEST RECORDED	REPLICATE X NUMBER SEEDS OF X WEIGHT (g)	TEMPERATURE (°C)	SUBSTRATE	PFE-TREATMENT	DAY OF FIRST COUNT	DAY OF FINAL COUNT	NUMBER OF SEED LOTS TESTED: NUMBER OF TESTS	COMMENTS
<i>Acacia oxycedrus</i>	38	36	37		4 x 100	25	TV 12cm dishes	BW*	7	35	1:1	*Pre-treatment used in test not recorded. recommend BW
<i>Acacia podalyriaefolia</i>	26-31	13	25	26	8 x 50	25	TV 12cm dishes	BW	7	35	2:2	
<i>Acacia prominens</i>	52	7	31		4 x 100	25	TV 12cm dishes	BW*	7	35	1:1	*Pre-treatment used in test not recorded. recommend BW
<i>Acacia richii</i>	39	26	27		4 x 100	25	TV 12cm dishes	BW*	7	35	1:1	*Pre-treatment used in test not recorded. recommend BW
<i>Acacia salicina</i>	15	9	14		8 x 50	25	TV 12cm dishes	BW	7	35	1:1	
<i>Acacia saligna</i> (A. cyanophylla)	48-58	12	49	52	4 x 100	25	TV 12cm dishes	BW	7	35	2:2	
<i>Acacia suaveolens</i>	81	74	74		4 x 100	25	TV 12cm dishes	BW*	7	35	1:1	*Pre-treatment used in test not recorded. recommend BW
<i>Acacia subulata</i>	28	6	19		8 x 50	25	TV 12cm dishes	BW*	7	35	1:1	*Pre-treatment used in test not recorded. recommend BW
<i>Acacia ulicifolia</i> (A. juniperina)	20-82	48	49	78	4 x 100	25	TV 12cm dishes	BW*	7	35	2:2	*Pre-treatment used in test not recorded. recommend BW
<i>Acacia uncinata</i> (A. undulifolia)	23	4	17		8 x 50	25	TV 12cm dishes	BW	7	35	1:1	
<i>Acacia vestita</i>	35	7	27		4 x 100	25	TV 12cm dishes	BW	7	35	1:1	
<i>Acacia victoriae</i>	18	11	13		8 x 50	25	TV 12cm dishes	BW	7	35	1:1	
<i>Aponis flexuosa</i>	a	675	745		4 x 0.15g	25	TP	None	7	21	1:1	
<i>Albizia lophantha</i>	9-16	11	13	14	4 x 100	25:20/30*	TV 12cm dishes	BW	10	21	5:7	*25°C gives quickest germination.
<i>Allocasuarina distyla</i> (Casuarina distyla)	1000	360	800		4 x 100	25	TPV	None	7	21	1:1	
<i>Allocasuarina littoralis</i> (Casuarina littoralis)	200-578	293	307	411	4 x 100	25:30*	TPV	None	5*	21*	10:10	*Used at Coffs Harbour. *Turnbull and Martens: (1982).

SPECIES	NUMBER OF SEEDS/G RANGE	NUMBER OF GERMINABLE SEEDS/G MEAN	NUMBER OF VIABLE SEEDS/G MEAN	NUMBER OF VIABLE SEEDS/G HIGHEST RECORDED	REPLICATE X NUMBER SEEDS OF X WEIGHT (g)	TEMPERATURE (°C)	SUBSTRATE	PRE-TREATMENT	DAY OF FIRST COUNT	DAY OF FINAL COUNT	NUMBER OF SEED LOTS TESTED: NUMBER OF TESTS	COMMENTS
<i>Allocasuarina torulosa</i> (<i>Casuarina torulosa</i>)	157-475	161	163	307	4 x 100	25±30#	TPV	None	5#	21#	0:0	#Used at Coffs Harbour, #Turnbull and Martensz (1982).
<i>Angophora costata</i>	63-79	59	61	67	4 x 100	25	TV 12cm dishes	None	5	21	3:3	
<i>Banksia aemula</i> (B. serratifolia)	10-13	7	7	7	4 x 100	25#	TV trays	None	14	35	9:9	#Heslehurst (1979) recommends 28-32°C.
<i>Banksia integrifolia</i> var. <i>integrifolia</i>	94	67	85		4 x 100	25#	TV 12cm dishes	None	14	35	1:1	#Heslehurst (1979) recommends 18-23°C.
<i>Banksia marginata</i>	125	109	109		4 x 100	25	TV 12cm dishes	None	14	35	1:1	
<i>Banksia serrata</i>	16-22	10	12	16	4 x 100	25#	TV trays	None	15	35	5:5	#Heslehurst (1979) recommends 20-28°C.
<i>Callistemon acuminatus</i>	a	2262	2262		4 x 0.04g	25	TP	None	6	21	1:1	
<i>Callistemon angustifolius</i>	a	1210	1320		4 x 0.07g	25	TP	None	6	21	1:1	
<i>Callistemon citrinus</i>	a	3107	3199	6625	4 x 0.03g	25	TP	None	6	21	5:6	
<i>Callistemon comboynensis</i>	a	2813	2857	3100	4 x 0.03g	25	TP	None	6	21	2:2	
<i>Callistemon linearifolius</i>	a	1105	1185		4 x 0.08g	25	TP	None	6	21	1:1	
<i>Callistemon linearis</i>	a	7244	7275	10767	4 x 0.01g	25	TP	None	6	21	8:10	
<i>Callistemon macropunctatus</i>	a	4337	4484	9370	4 x 0.02g	25	TP	None	6	21	6:7	
<i>Callistemon paludosus</i>	a	4155	4230		4 x 0.02g	25	TP	None	6	21	1:1	
<i>Callistemon phoeniceus</i>	a	4701	4735	11135	4 x 0.02g	25	TP	None	6	21	6:6	
<i>Callistemon pinifolius</i>	a	4785	4835		4 x 0.02g	25	TP	None	6	21	1:1	
<i>Callistemon rigidus</i>	a	3183	3204	7312	4 x 0.03g	25	TP	None	6	21	6:6	
<i>Callistemon salignus</i>	a	1508	1524	2788	4 x 0.06g	25	TP	None	6	21	8:9	
<i>Calothamnus chrysantherus</i>	a	190	290		4 x 0.50g	25	TP	None	8	35	1:1	
<i>Calothamnus gilesii</i>	a	175	658		4 x 0.15g	25	TP	None	8	35	1:1	High % ungerminated seeds, other temperatures may be more successful.

SPECIES	NUMBER OF SEEDS/6 RANGE	NUMBER OF GERMINABLE SEEDS/6 MEAN	NUMBER OF VIABLE SEEDS/6 MEAN	NUMBER OF VIABLE SEEDS/6 HIGHEST RECORDED	REPLICATE X NUMBER SEEDS OF X WEIGHT (g)	TEMPERATURE (°C)	SUBSTRATE	PRE-TREATMENT	DAY OF FIRST COUNT	DAY OF FINAL COUNT	NUMBER OF SEED LOTS TESTED: NUMBER OF TESTS	COMMENTS
<i>Calothamnus quadrifidus</i>	a	153	700		4 x 0.15g	25	TP	None	8	35	1:1	High % ungerminated seeds, other temperatures may be more successful.
<i>Calothamnus sanguineus</i>	a	405	968		4 x 0.10g	25	TP	None	8	35	1:1	High % ungerminated seeds, other temperatures may be more successful.
<i>Calothamnus villosus</i>	a	380	457		4 x 0.20g	25	TP	None	6	35	1:2	Light for more than 8 hours per day resulted in higher germination %.
<i>Casuarina decasneana</i>	66	42	42		4 x 100	25† 30:35†	TPV 12cm dishes	None	5†	14†	1:1	†Used at Coffs Harbour. †Turnbull and Martensz (1982). †ISTA (1985): Boland, Brooker and Turnbull (1980).
<i>Eucalyptus</i> spp.	†	†	†	†	†	†	†	†	†	†		
<i>Grevillea robusta</i>	91-200	69	69	80	4 x 100	25	TV 12cm dishes	CW 48hr	7	21	2:2	
<i>Hakea salicifolia</i>	64-100	61	63	100	4 x 100	25†	TV 12cm dishes	None	16	35	4:4	†20/30°C resulted in 10% lower germination and less than 50% viability in one test.
<i>Leptospermum flavescens</i>	a	1413	1428	2430	4 x 0.07g	25	TP	None	7	21	4:4	
<i>Leptospermum juniperinum</i>	a	240	365		4 x 0.25g	25	TP	None	7	21	1:1	
<i>Leptospermum laevigatum</i>	a	1720	1735		4 x 0.06g	25	TP	None	7	21	1:1	
<i>Leptospermum petersonii</i>	a	825	836	1538	4 x 0.10g	25	TP	None	7	21	2:2	
<i>Leptospermum scoparium</i> var. <i>scoparium</i>	a	1020	1035		4 x 0.10g	25	TP	None	7	21	1:1	
<i>Lophostemon confertus</i> (<i>Tristania conferta</i>)	a	359	360	550	4 x 0.25g	25	TP	None	5	21	11:13	
<i>Melaleuca alternifolia</i>	a	4375	5175		4 x 0.02g	25	TP	None	7	28	1:1	
<i>Melaleuca arvensis</i>	a	2277	2365	3180	4 x 0.04g	25	TP	None	7	28	2:3	
<i>Melaleuca bracteata</i>	a	5198	6028	9715	4 x 0.01g	25	TP	None	7	28	2:2	
<i>Melaleuca decussata</i>	a	3995	4110	4525	4 x 0.02g	25	TP	None	7	28	2:3	
<i>Melaleuca halimaturorum</i>	a	1790	1815		4 x 0.05g	25	TP	None	7	28	1:1	
<i>Melaleuca hugelii</i>	a	20	285		4 x 0.30g†	25	TP	None	7	28	1:1	†This replicate weight is high compared to that for other <i>Melaleuca</i> species. It may not be suitable for other seed lots of this species.
<i>Melaleuca hypericifolia</i>	a	3211	3654	7250	4 x 0.02g	25	TP	None	7	28	7:7	

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SPECIES	NUMBER OF SEEDS/G RANGE	NUMBER OF GERMINABLE SEEDS/G MEAN	NUMBER OF VIABLE SEEDS/G MEAN	NUMBER OF VIABLE SEEDS/G HIGHEST RECORDED	REPLICATE X NUMBER SEEDS OF X WEIGHT (g)	TEMPERATURE (°C)	SUBSTRATE	PRE-TREATMENT	DAY OF FIRST COUNT	DAY OF FINAL COUNT	NUMBER OF SEED LOTS TESTED: NUMBER OF TESTS	COMMENTS
<i>Melaleuca incana</i>	a	720	723	725	4 x 0.13g	25	TP	None	7	28	2:2	
<i>Melaleuca lanceolata</i>	a	1437	1468	1785	4 x 0.07g	25	TP	None	7	28	3:3	
<i>Melaleuca laterita</i>	a	6407	6667		4 x 0.01g	25	TP	None	7	28	1:1	
<i>Melaleuca laxiflora</i>	a	2745	3340		4 x 0.03g	25	TP	None	7	28	1:1	
<i>Melaleuca linariifolia</i>	a	5874	6038	11640	4 x 0.01g	25	TP	None	7	28	4:4	
<i>Melaleuca parvifolia</i>	a	785	975		4 x 0.08g	25	TP	None	7	28	1:1	
<i>Melaleuca quinquinervia</i>	a	3269	3305	4945	4 x 0.03g	25	TP	None	7	28	4:4	
<i>Melaleuca radula</i>	a	1705	1810		4 x 0.05g	25	TP	None	7	28	1:1	
<i>Melaleuca styphelioides</i>	a	1710	1740		4 x 0.06g	25	TP	None	7	28	1:1	
<i>Melaleuca viminalis</i> (<i>Callistemon viminalis</i>)	a	1320	1334	1945	4 x 0.07g	25	TP	None	6	21	3:4	
<i>Syncarpia glomulifera</i>	a	113	118	158	4 x 0.80g	25	TP	None	6	21	5:7	
<i>Xanthorrhoea johnsonii</i>	77-89	68	69	76	4 x 100	25	TV 12cm dishes	None	14	28	4:4	

TABLE 2. RESULTS OF GERMINATION TESTS AND METHODS USED FOR SPECIES WHICH MAY HAVE A LARGE PROPORTION OF EMPTY SEED

SPECIES	NUMBER OF SEEDS/G RANGE	NUMBER OF GERMINABLE SEEDS/G MEAN	NUMBER OF VIABLE SEEDS/G MEAN	NUMBER OF VIABLE SEEDS/G HIGHEST RECORDED	REPLICATE X NUMBER SEEDS OR X WEIGHT (g)	TEMPERATURE (°C)	SUBSTRATE	PRE-TREATMENT	DAY OF FIRST COUNT	DAY OF FINAL COUNT	NUMBER OF SEED LOTS TESTED; NUMBER OF TESTS	COMMENTS
<i>Allocasuarina verticillata</i> (<i>Casuarina stricta</i>)	313	72	140		4 x 100	25:30†	TPV	None	7†	28†	1:1	†Turnbull and Martensz (1982): 25°C used at Coffs Harbour.
<i>Atriplex nummularia</i>	109-224	9	56	169	4 x 100	25	TPV	CW†	7	21	4:6	†Beadle (1952) recommends that after soaking, all excess water be removed by squeezing fruits between pieces of blotting paper.
<i>Callitris columellaris</i> (<i>C. glauca</i> , <i>C. hugelii</i>)	51-96	7	8	20	4 x 100	20†	TV	None	17	35	6:6	†Scott (1970). There are often many empty seeds in <i>Callitris</i> spp. Scott (1970) reports 5-40% viability in even freshly collected seed.
<i>Callitris endlicheri</i>	119-166	0	1	1	4 x 100	20†	TV	None	14?	35	2:2	†Scott (1970).
<i>Callitris rhomboidea</i>	158	45	46		4 x 100	25	TV	None	14	35	1:1	
<i>Casuarina cristata</i>	178-311	24	25	92	4 x 100	25†	TPV	None	7†	21†	4:4	†Turnbull and Martensz (1982).
<i>Casuarina cunninghamiana</i>	1508-2140	353	360	1240	4 x 100	25:30:35†	TPV	None	5†	21†	4:4	†Turnbull and Martensz (1982): 25°C used at Coffs Harbour.
<i>Casuarina equisetifolia</i> var. <i>incana</i>	489	205	205		4 x 100	20:25:30†	TPV	None	7†	21†	1:1	†Turnbull and Martensz (1982): 25°C used at Coffs Harbour.
<i>Casuarina glauca</i>	1064-2178	194	223	720	4 x 100	20:25†	TPV	None	7†	24†	6:7	†Turnbull and Martensz (1982): 25°C used at Coffs Harbour.

TABLE 2. RESULTS OF GERMINATION TESTS USING METHODS CONSIDERED TO BE UNRELIABLE

SPECIES	NUMBER OF SEEDS/G RANGE	NUMBER OF GERMINABLE SEEDS/G MEAN	NUMBER OF VIABLE SEEDS/G MEAN	NUMBER OF VIABLE SEEDS/G HIGHEST RECORDED	REPLICATE X NUMBER SEEDS OF X WEIGHT (g)	TEMPERATURE (°C)	SUBSTRATE	PRE-TREATMENT	DAY OF FIRST COUNT	DAY OF FIRST COUNT	NUMBER OF SEED LOTS TESTED; NUMBER OF TESTS	COMMENTS
<i>Brachychiton acerifolius</i>	3-4	0	1	3	4 x 100	25	TV trays	None†	10 th	28	3:5	†HW, BW and S pre-treatments all unsuccessful. S resulted in many dead seeds. Low germination† resulted when no pre-treatment was used.
<i>Brachychiton populneus</i>	7-10	1	3	9	4 x 100	25	TV trays	None†	10 th	28	4:5	†S pre-treatment resulted in many dead seeds. Nil germination and high % ungerminated seeds resulted when no pre-treatment was used.
<i>Cassia artemesioides</i>	61-100	2	2	4	4 x 100	25	TV	HW†	7	21	2:2	†HW and BW pre-treatments both resulted in high % dead seeds. HW resulted in lower. Other pre-treatments such as CW or a warm water soak (say, 40-50 C) could be tried.
<i>Cassia barclayana</i> var. <i>barclayana</i> (<i>C. sophora</i>)	63-78	5	5	9	4 x 100	25	TV	HW†	7	21	2:2	†See note for <i>C. artemesioides</i> .
<i>Cassia eremophila</i>	33-67	4	9	27	4 x 100	25	TV	HW†	7	21	5:5	†See note for <i>C. artemesioides</i> .
<i>Cassia notabilis</i>	37	17	27		4 x 100	25	TV	HW†	7	21	1:1	†See note for <i>C. artemesioides</i> .
<i>Cassia odorata</i> (<i>C. australis</i>)	61	0	41		4 x 100	25	TV	HW†	7	21	1:1	†See note for <i>C. artemesioides</i> .
<i>Cassia sturtii</i>	100	6	6		4 x 100	25	TV	HW†	7	21	1:1	†See note for <i>C. artemesioides</i> .
<i>Hakea laurina</i>	37-43	0	23	25	4 x 100	25	TV 12cm dishes	CW†	?	?	2:2	†Both tests resulted in over 50% ungerminated seed with no pre-treatment. Cold water soak suggested.
<i>Hakea petiolaris</i>	13	6	9		4 x 100	25	TV 12cm dishes	CW†	21	42	1:1	†Over 25% ungerminated seed and slow germination with no pre-treatment. Cold water soak suggested.

