



Smoke Germination of Australian Plants

**A report for the Rural Industries Research
and Development Corporation**

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October 1998

RIRDC Publication No 98/108
RIRDC Project No KPW-1A

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ISBN 0 642 57855 9
ISSN 1440-6845

"Smoke germination of Australian plants"
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Project no. KPW-1A

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Published in October 1998
Printed on environmentally friendly paper by the DPIE Copy Centre

Foreword

Understorey species provide critical components in most landscapes in Australia, yet methods to propagate, restore and manage are often limited by inadequate knowledge of the methods for species recruitment from seed.

The discovery that smoke plays a critical role in germinating seeds of Australian native species has opened up new opportunities for restoring, managing and growing our native plants.

This publication details the development and application of smoke for nursery propagation with opportunities for the use of smoke as a tool for land rehabilitation and management.

Until now, no simple process or method, other than the use of fire with concomitant deleterious environmental effects has been as universal as smoke in eliciting a widespread and uniform germination response in native species.

This report forms part of RIRDC's Wildflowers & Native Plants R&D program, which aims to improve the profitability, productivity and sustainability of the Australian wildflower and native plant industry.

Peter Core

Managing Director

Rural Industries Research and Development Corporation

Acknowledgments

Shauna Roche provided inspiration throughout the project while many nursery persons, wildflower farmers, seed merchants and wildflower enthusiasts were instrumental in assisting with provision of field sites, seeds and support. Bob Dixon provided outstanding horticultural advice and guidance throughout the project. Keran Keys and horticultural support staff of the Division of Living Collections and Natural Heritage provided practical support in maintenance of the research materials used in this study. Anle Tieu and Ingrid Sieler must also be acknowledged as key supporters throughout this project.

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EXECUTIVE SUMMARY

USING SMOKE TO GERMINATE AUSTRALIAN NATIVE PLANTS

Following a lead from South African botanists, scientists at Kings Park and Botanic Garden in Western Australia have found that it is not the heat and ash from a fire, but rather the smoke that holds the key to germination for many native Australian plants.

Smoke is now widely used in nursery production, bushland management and mine-site restoration.

It is now possible to use smoke at home, on the farm or in the nursery to germinate native plants, as summarized below.

A companion CD-ROM is available which describes in detail many of the practical uses of smoke (see below for ordering information).

What Species Can be Grown?

Over 400 native species of seeds respond to smoke treatment.

Smoke can be applied as water (i.e. smoke water) or in the aerosol form to seed trays, bushland soil or directly applied to seeds.

Research has found that smoke responsive native species occur throughout temperate southern and arid Australia. Even species from habitats which are not fire-prone (e.g. the alpine herb fields of Tasmania) germinate well following application of smoke. Tropical species may require research to determine the extent to which smoke may be important for their germination.

Benefits of Smoke

Whereas heat and ash are of limited value for breaking dormancy in many species, smoke promotes:

- Germination of species which are difficult to germinate by conventional means
- More uniform germination
- Earlier germination
- Seedlings which are more robust.

- **Which Plants Respond to Smoke?**

Trees, shrubs, herbs and annuals respond to smoke-treatment.

There is no clear distinction in the relationship between taxonomic groups and the requirement for smoke. Proteaceae, Myrtaceae and most other dominant Australian plant families contain smoke responsive species. Some experimentation is essential to determine if an untested species might be smoke responsive. Do not rely only on evidence from related species to predict if a species is smoke responsive.

When to Smoke

For regions in southern Australia, smoke is best applied from autumn to early winter. For tropical or arid zone species, some experimentation may be required to determine the best time to apply smoke. As a general rule, sowing and smoking should be done when germination is most likely to occur in nature.

Smoke is highly water soluble and excessive watering of seed trays can leach the active agents from the soil before seed dormancy has been broken.

How to Make and Use Smoke

Liquid (smoke water) or aerosol smoke are the two most common methods for applying smoke to soil or seeds.

Nursery Propagation

Seeds can be smoked directly in a smoke tent or alternatively soaked in a dilute solution of smoke water (see below for method) for 6-24 hours. Treated seeds are then dried and sown when required. Alternatively, trays containing sown seeds can be smoked for 60 minutes and then carefully watered for the first 6-10 days to ensure adequate penetration of the smoke chemicals.

Soil Restoration/Bushland Management

Broadcast seed which has been smoke treated is an effective way to germinate a wide variety of species. Smoke treated seeds used in broadcasting often germinate better including seeds of species which do not normally require smoke for germination under nursery conditions (e.g. Eucalypts, Banksias).

If a seed bank is present in soil then good germination is possible following the addition of smoke either using aerosol smoke (area for treatment is limited using this method) or smoke water (using automated sprayers).

Making Your Own Smoke & Smoke Water

A smoke tent with smoke generator, cooling pipe and inlet fan can be used to produce aerosol smoke for treating seed. The tent can contain up to three levels of shelving. Leave approximately 30 cm between each shelf to ensure adequate flow of smoke between the shelves.

Smoke water is produced by drawing smoke from smoke generator through drums (20-30 L) containing water for up to 60 minutes.

What to Burn

Most types of fresh or dried plant or woody materials will produce potentially useful smoke. Good germination results are obtained when a mixture of dry and green foliage and twigs are burnt. Plants with white sap (e.g. Euphorbiaceae) or oleander should not be combusted as the smoke produced may be noxious.

Plant Genera that are Responsive to Smoke

<i>Acacia</i> *	<i>Diplolaena</i>	<i>Myriocephalus</i>
<i>Acanthocarpus</i>	<i>Drosera</i>	<i>Neurachne</i>
<i>Acrotriche</i>	<i>Epacris</i>	<i>Opercularia</i> *
<i>Actinostrobos</i>	<i>Eriostemon</i>	<i>Orthrosanthus</i>
<i>Actinotus</i>	<i>Eucalyptus</i> *	<i>Patersonia</i>
<i>Adenanthos</i> *	<i>Exocarpus</i>	<i>Persoonia</i>
<i>Agonis</i>	<i>Gahnia</i>	<i>Petrophile</i>
<i>Agrostocrinum</i>	<i>Geleznovia</i>	<i>Phyllanthus</i> *
<i>Allocasuarina</i> *	<i>Georgiella</i>	<i>Pimelea</i>
<i>Alyxia</i>	<i>Gompholobium</i> *	<i>Pityrodia</i>
<i>Amphipogon</i>	<i>Gonocarpus</i>	<i>Platysace</i>
<i>Andersonia</i>	<i>Grevillea</i>	<i>Pomaderris</i>
<i>Anigozanthos</i>	<i>Gyrostemon</i>	<i>Poranthera</i> *
<i>Arthropodium</i>	<i>Haemodorum</i>	<i>Ptilotus</i>
<i>Astartea</i>	<i>Hakea</i>	<i>Ricinocarpus</i>
<i>Astroloma</i>	<i>Hemigenia</i> *	<i>Rulingia</i>
<i>Baeckea</i>	<i>Hemiphora</i>	<i>Scaevola</i>
<i>Banksia</i> *	<i>Hibbertia</i>	<i>Siegfriedia</i>
<i>Billardiera</i>	<i>Hovea</i> *	<i>Sollya</i>
<i>Blancoa</i>	<i>Hyalosperma</i> *	<i>Sowerbaea</i> *
<i>Boronia</i>	<i>Hybanthus</i>	<i>Sphenotoma</i>
<i>Bossiaea</i> *	<i>Hydrocotyle</i> *	<i>Spyridium</i>
<i>Brunonia</i>	<i>Hypocalymma</i>	<i>Stackhousia</i>
<i>Burchardia</i>	<i>Isopogon</i>	<i>Stipa</i> *
<i>Bursaria</i>	<i>Isotoma</i> *	<i>Stirlingia</i>
<i>Caesia</i>	<i>Johnsonia</i>	<i>Stylidium</i>
<i>Callitris</i>	<i>Kennedia</i> *	<i>Tersonia</i>
<i>Calytrix</i>	<i>Lachnostachys</i>	<i>Tetragia</i>
<i>Chamaescilla</i>	<i>Lasiopetalum</i>	<i>Tetrarrhena</i>
<i>Chieranthera</i>	<i>Laxmannia</i>	<i>Tetratheca</i>
<i>Clematis</i>	<i>Lechenaultia</i>	<i>Thysanotus</i>
<i>Codonocarpus</i>	<i>Leptomeria</i>	<i>Trachymene</i> *
<i>Comesperma</i>	<i>Leptospermum</i>	<i>Trichocline</i>
<i>Conospermum</i>	<i>Leucopogon</i>	<i>Tripterococcus</i>
<i>Conostephium</i> *	<i>Levenhookia</i> *	<i>Trymalium</i> *
<i>Conostylis</i>	<i>Lobelia</i>	<i>Velleia</i>
<i>Crassula</i>	<i>Lomandra</i>	<i>Verticordia</i>
<i>Cryptandra</i>	<i>Loxocarya</i>	<i>Waitzia</i> *
<i>Cyathochaeta</i> *	<i>Lysinema</i>	<i>Xanthorrhoea</i> *
<i>Dampiera</i> *	<i>Macropidia</i>	<i>Xanthosia</i>
<i>Desmocladius</i> *	<i>Melaleuca</i> *	
<i>Dianella</i>	<i>Mitrasacme</i>	

*Australian genera which are responsive to smoke and germinate under nursery or field conditions

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CHAPTER 1. GENERAL INTRODUCTION

1.1 INTRODUCTION

The Australian flora is world renown for its beauty and exotic forms and is becoming more widely desired for use within amenity horticulture and the cut flower industry. Australian native plant production is worth about \$85m annually (1996), however, this represents only 10% of the world production of Australian native flowers (Flower Export Council of Australia Inc. 1997). The development of new crop species from our diverse flora will assist Australian growers in occupying a greater market share. Added to this are existing and new prospects for essential oil industries, glycoprotectants and resins and pharmacological products from native species which will require efficient production of greenstock.

Presently about 20% of the native plant product, representing at least 200 species, is sourced from wild populations. The propagation of these commercially significant native species is limited to sporadic or erratic seed germination or costly *in vitro* procedures, leaving collection from native stands as the only economic production methods available for cutflower and foliage. With the rapid growth of the industry, increased pressure will be placed on natural populations which can only be detrimental to these species.

The challenge is to develop new germination strategies to promote cost effective and efficient means for large scale propagation of key taxa. The primary factors limiting the cultivation of these species have been, low seed set, poor seed quality but, most importantly, seed dormancy.

In recent years, smoke (and smoke products) has been implicated as a key agent in the promotion of germination in over 40 species of previously highly recalcitrant native Western Australian plants. Overlooked as an agent for germination, aerosol smoke or smoke in an aqueous phase is capable of germinating seeds in nursery and habitat situations. Research and development of this new and exciting procedure promises a simple and effective process for propagation of many Australian plants which show no response or which only have very low germination rates using conventional procedures.

The key taxa which have been found in preliminary studies by the applicant to respond to smoke stimulation include species in the genera *Verticordia*, *Eriostemon*, *Geleznowia*, *Grevillea*, *Anigozanthos*, *Stirlingia*, *Conospermum* and *Hibbertia*. Members of the Epacridaceae (eg *Lysinema*, *Leucopogon*) have also responded in a similar manner. These plant groups alone comprise a significant and outstanding group of wild sourced species which account for at least 6 million stems cut annually from native stands in Western Australia and worth approximately \$1.25 million (1993).

Facilitation of germination in some species will have an immediate impact on the horticultural practise and commercial benefits arising from those species. For example, development of germination methods for the disturbance opportunistic genus *Actinotus* (flannel flowers) could significantly enhance sales of premium product. The application of smoke technology to the *Actinotus* industry currently under supplied nationally with good quality stems would pay dividends rather quickly as could be said of a range of species with similar germination responses. For some species (eg *Stirlingia latifolia* and *Geleznowia verrucosa*, two of the most significant wild picked species in Western Australia) smoke technology also provides the first cost effective alternative to costly tissue culture methods of production and could mean immediate introduction into production horticulture of established and currently, highly profitable species.

The introduction of new species into cultivation has been limited by a few factors, including difficulties in accessing natural populations, low seed set, poor seed quality and seed dormancy. Of these seed dormancy is most probably the primary limiting step in the development of native species for horticulture.

Breaking seed dormancy remains the most important factor in improving seed germination of Australian native species (Richards and Beardsell, 1987; Bell *et al.*, 1993). Dormancy mechanisms are often adaptations to the mediterranean and semi arid climates and their associated frequent fires which occur in Australian habitats (Bell *et al.*, 1993; Richards and Beardsell, 1987).

Burning of habitats is the most important trigger of natural germination events in temperate Australian ecosystems (Gill 1981; Bell *et al.* 1993). Heat from fire has been found to trigger

germination in hard-seeded species by breaking seed-coat imposed dormancy and in serotinous species by fostering the release of canopy-stored seed (Fordham 1968; Bradstock 1991; Lamont *et al.* 1991). A much more prevalent germination response in Australian native seeds is that induced by exposure to smoke. Dixon *et al.* (1995) found that the seed of 23 species of Australian plants, which had previously been difficult to germinate using conventional techniques, responded positively to smoke stimulation. The number of Australian species known to be smoke responsive species is now close to 200 (Roche 1997, unpubl. thesis). Smoke enhanced germination response transcends taxonomic groups (Angiosperms: monocotyledons and dicotyledons, and Gymnosperms), life forms (trees to herbs), and reproductive strategies (annual seeders, bradysporous species and long-lived resprouters) and a large number of plant families (over 40 families) (Roche *et al.* 1997b). The discovery of smoke as a propagation tool has dramatically increased the number of genera that can be propagated using a relatively simple technique, however many species are still difficult to propagate from seed and the presence of long term dormancy mechanism precludes their introduction into horticulture.

1.2 SEED GERMINATION AND DORMANCY

1.2.1 Physical requirements for germination

If a seed does not germinate in suitable conditions which include conducive temperature, atmospheric composition and water availability (Bradbeer, 1983; Mayer and Poljakoff-Mayber, 1989; Bewley and Black, 1995), the seed must either be dead (not viable) or viable but in a dormant state. Breaking seed dormancy remains the most important factor in improving seed germination of Australian native species (Richards and Beardsell, 1987; Bell *et al.*, 1993).

1.2.2 Seed viability

Australian seed often has low viability contributing to low germination. Out of 73 south western Australian species, only 51 % were found to be viable using tetrazolium stain testing (Bell *et al.*, 1993). Analysis of germination needs to take this into account. There are various methods to determine seed viability, ranging from the simple observation of endosperm

presence to the more difficult embryo rescue (Bradbeer, 1983; Mayer and Poljakoff-Mayber, 1989).

1.2.3 Overview of dormancy mechanisms

A dormant seed is defined as a viable seed that does not germinate under favourable conditions (Mayer and Poljakoff-Mayber, 1989; Bewley and Black, 1995). There are two states of dormancy: primary dormancy, when seeds become dormant while still on the parent plant; and secondary dormancy, which is induced in mature and non dormant seeds by unfavourable conditions (Bewley and Black, 1995). Mechanisms by which dormancy is imposed can be classified into two groups:

- i seed coat imposed
- ii embryo imposed

Both dormancy mechanisms are based on either the inability of the embryo itself to grow or tissues enclosing the embryo preventing its growth (Bewley and Black, 1995).

Seed coats can impose dormancy in several ways. These include restricting gas exchange, water uptake, mechanically restricting the embryo, presence of chemical inhibitors and failure to mobilise embryo food reserves (Bradbeer, 1988). Embryo imposed dormancy may be due to poor embryonic development, blocks to nucleic acid and protein synthesis, failure to mobilise food reserves and presence of inhibitors. Dormancy in Australian plant species can operate by seed coat or embryo dormancy or both. Examples include mechanical restriction of the coat in *Acacia* species (Mott and Groves, 1981) and endosperm or embryo inhibitors in *Lomandra sonderi* (F.Muell.) (Plummer *et al.*, 1995). Elucidating dormancy mechanisms of Australian plant species is therefore a complex task.

1.2.4 Multiple dormancy mechanisms and staggered germination

Seed dormancy is often controlled by a series of factors which control interactive processes that lead to inhibition of germination (Champagnat, 1983). For many Western Australian species it is unlikely that 100 % germination can be achieved with a single treatment as there is usually more than one dormancy mechanisms in action (Dennis, 1994). Multiple dormancy

mechanisms may require two or more environmental cues working in combination or sequentially to break dormancy (Bell *et al.*, 1993). For example, in Western Australian *Acacia* species, heat shock followed by correct temperatures is required for optimum germination (Bell and Bellairs, 1992)

1.3 A ROLE FOR SMOKE MEDIATED GERMINATION IN HORTICULTURE

A major breakthrough in overcoming dormancy in Australian plant species was the discovery that smoke derived from the combustion of plant material could positively affect the seed germination of a wide range of species. De Lange and Boucher (1990) found that combustion of plant material and smoke, used in aqueous or aerosol form, enhanced seed germination in *Audouinia capitata*, a fire-following and threatened South African fynbos species. Since that discovery, over 170 species native to mediterranean-type ecosystems on three continents have been shown to be similarly affected (Brown 1993a; Brown *et al.* 1993; Baldwin *et al.* 1994; Baxter *et al.* 1994; Brown *et al.* 1994b; Brown *et al.* 1995; Dixon *et al.* 1995; Pierce *et al.* 1995; Sutcliffe *et al.* 1995; C. J. Fotheringham pers. comm.). This diverse group of species includes members of 37 families and 88 genera representing gymnosperms, monocotyledons and dicotyledons, as well as species described as those which demonstrate both the seeder and resprouter modes of recovery after fire. It also embraces a range of plant forms from geophytes to phanerophytes. Furthermore, smoke-derived products have been shown to break the dormancy of two common vegetable species (Drewes *et al.* 1995; Thomas and van Staden 1995).

A wide range of Australian species have been found to respond positively to smoke treatment. Especially exciting was the dramatic improvement in the germination of species that had previously been particularly difficult to germinate (Roche, 1993; Dixon *et al.*, 1995). Improvements in germination of 40-100% for germinable species (including in the Haemodoraceae, Goodeniaceae, Liliaceae) and up to 400% for recalcitrant species in the genera *Verticordia*, *Eriostemon*, *Geleznowia*, *Grevillea*, *Anigozanthos*, *Stirlingia*, *Conospermum*, Epacridaceae (eg *Lysinema*, *Leucopogon*) and *Hibbertia* have been achieved.

1.3.1 Active principles in smoke

Smoke's role in breaking dormancy is only a recent discovery and the exact mechanism by which smoke breaks dormancy is unclear. Baldwin *et al.* (1994) proposed that smoke interacts chemically with inhibitors in the seed coat, endosperm or embryo to enhance seed germination, and that stimulation of germination is due to smoke-specific signal molecule(s), possibly promotive hormones.

In a recent study, Egerton-Warburton (1997, unpubl.) proposed that volatiles within smoke may act in a manner analogous to surfactants to break seed dormancy in *Emmenanthe penduliflora*, a South African species which demonstrates a prolonged seed dormancy followed by germination closely cued to the immediate post-fire environment. Short exposure to smoke (3 minutes) was found to promote a significant increase in seed germination (dormant $8\pm 0.3\%$; smoke-treated $79\pm 3\%$). Exposure to smoke resulted in 2 major changes to the morphology of the seed. Firstly, it scarified the seed surface overcoming the primary resistance to the diffusion of water into the seed, enforced by a cuticular layer between the testa and endosperm. Secondly, smoke altered the permeability of the internal (subtesta) cuticle which may act as a barrier against the outward release of endogenous inhibitors from the seed proper (resulting in prolonged dormancy).

1.3.2 Methods for applying smoke for germination

There are various methods to treat seeds with smoke. De Lange and Boucher (1990) are credited with first using aerosol and aqueous smoke to initiate seed germination using South African flora. In Australian species, both smoked water and aerosol smoke have been shown to improve germination (Dixon *et al.*, 1990; Roche, 1993). After discovering smoke's merits, the natural progression is to find commercially viable and effective methods to treat difficult to germinate seeds of species which have economic potential. Three effective ways have been developed by which smoke can be applied to seed:

a) Application of aerosol smoke to seed trays;

Seeds are sown in punnets and placed into a sealed, steel-framed plastic tent measuring 2x1.5x1m. Smoke is generated in a 60-litre steel drum by slow combustion of organic materials. The type of plant material from which smoke is derived is not particularly

important in generating the final product however generally a mixture of fresh and dry native plant leaf and stems is generally used. The drum is fitted with an inlet through which air is pumped at the rate of 30-50L/min, and the resulting smoke is cooled as it passes through a 2-m length of flexible plastic hosing before passing into the fumigation tent containing the seeds sown in punnets. After 60 minutes in the smoke tent, punnets are transferred to the glasshouse and gently handwatered for the first week to ensure the smoke chemical is not washed out of the punnets. Smoked soil surfaces emit a distinctive smoked odour which lasts for 6-8 days.

b) Direct application of aerosol smoke to seed; and

Aerosol smoke, generated using the smoke tent method (described above), can also be directly applied to seed. The general method now used is to place the seed on an open tray in a smoke tent for one hour. The seed can then be either stored indefinitely or sowed directly.

c) Application of smoke water to seed trays and smoke water imbibition of seed.

Smoked water is produced by bubbling smoke from a 60-litre steel combustion drum through 100 litres of water for 60 minutes. A mix of fresh and dry native plant cuttings are used as fuel. Twelve hundred litres of smoked water can be produced using this batch system. The optimum concentration of smoke water for germination is generally 10% of full strength. Seeds are imbibed in 10% smoke water for 3-6 hours or overnight. Once the smoke water is prepared it can be frozen and stored for up to 2 years.

1.4 BIOASSAY SPECIES

In order to test the mode of action of germination enhancement by smoke in native seed it was necessary to develop a rapid bioassay to expedite evaluation of results. A bioassay can be defined as a test that uses a biological indicator to evaluate a hypothesis.

The major criteria required for selecting a species with seed that could be used in a bioassay were:

i a highly significant germination response with smoke as compared with water (the control)

i consistency and reliability in the germination response to smoke as compared to the control
ii a species whose seed could be obtained in substantial amounts to allow continuity of use in smoke analysis

To determine useful test species for smoke experiments a broad suite of native species were trialed (Dixon *et al.* 1995).

Aerosol smoke was applied for one hour to punnets containing seed. Following exhaustive evaluation of a broad range of smoke responsive species, *Lysinema ciliatum* and *Stylidium affine* were selected for detailed evaluation as bioassay species.

Lysinema ciliatum was initially selected for bioassays as smoke promoted 44% germination in treated seed compared to less than 10% in the controls (Dixon *et al.* 1995). Unfortunately, the germination of control treatments increased with time in storage. This ageing phenomenon was not stabilized and the evaluation of this species for bioassaying was not continued.

Stylidium affine was an excellent species for use in a bioassay as it responded well to smoke with no germination in the controls. The germinants were easy to count, the germination percentage was representative of final germination after 3-6 weeks incubation and bulk quantities of seed are available. In addition, the species has proven to be the most reliable indicator under a wide variety of glasshouse and controlled climate (incubator) conditions and consistently yields germination after smoke treatment.

A more rapid bioassay was developed by Drewes *et al.* (1995) using *Lactuca sativa* L. (lettuce) seed (cultivar Grand Rapids). Plant-derived smoke replaces the light requirement normally required to break dormancy. Germination is carried out in the dark. The benefit of using this species is that it takes only 2 days for a germination result compared to 3-6 weeks for *Stylidium affine*. Lettuce provided a reliable and rapid germination response, however the species proved to be erratic in response to repeatability with variable germination responses depending on slight changes in environmental conditions. Further work on this species was not considered appropriate.

Following extensive testing and evaluation *Stylidium affine* was chosen as the most reliable species for use as a bioassay.

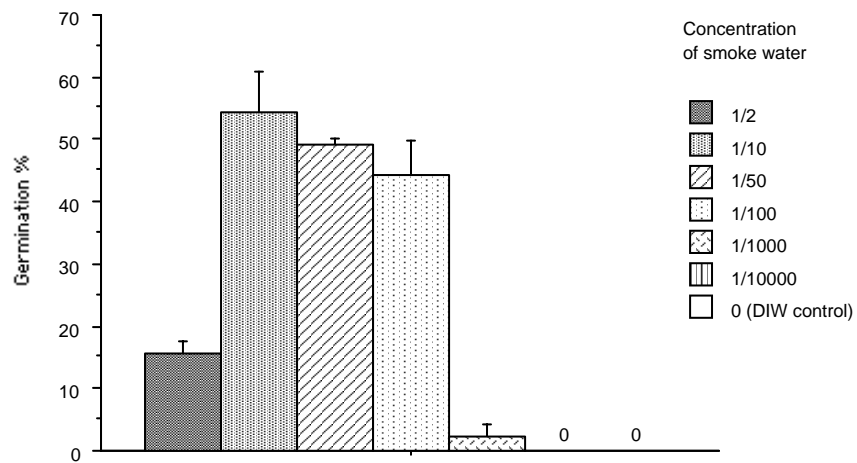
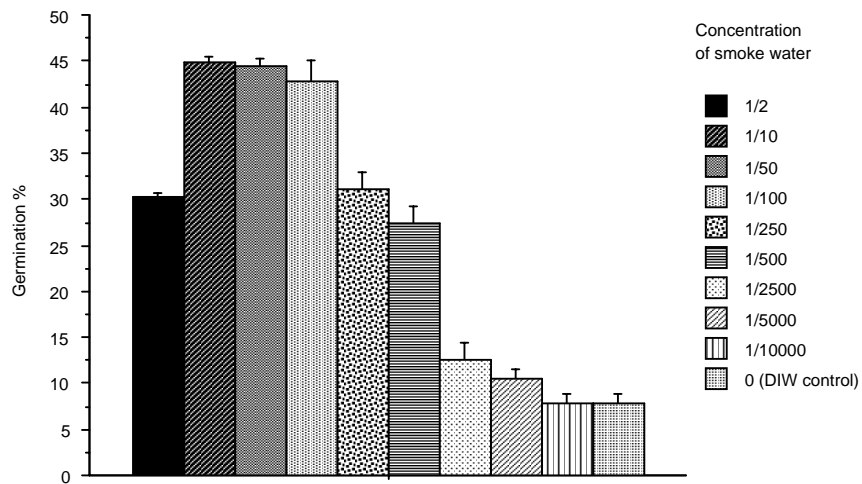


Fig. 1.1—Germination levels for *Lysinema ciliatum* (above) and *Styliidium affine* (below) seed with dilutions of smoke water (control is distilled water). Germination was scored at 6 weeks.

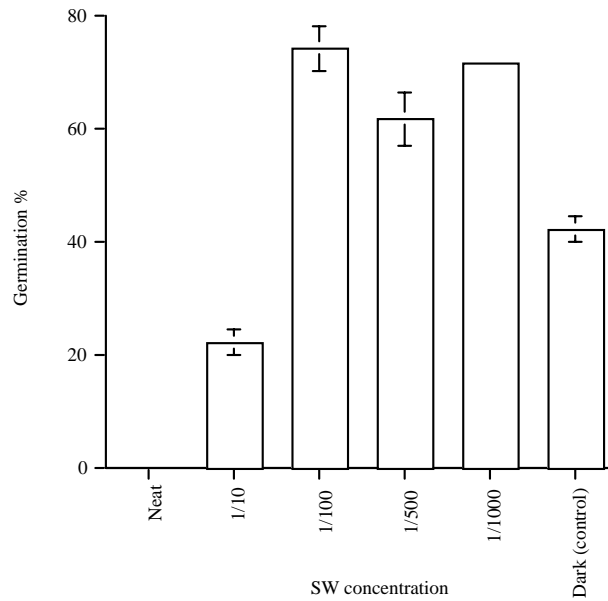


Fig. 1.2—Germination levels for Grand Rapids lettuce seed with dilutions of smoke water (control is distilled water). Germination was scored at 6 weeks.

1.5 OVERALL OBJECTIVE

The overall objective of this research programme is to develop smoke germination technology for commercial horticultural development of Australian species recalcitrant to conventional seed propagation methods which will minimize the environmental threats of wild collection of native species. This objective can be expressed as two main research directives.

1.5.1 Directive 1

The first directive aimed to increase our understanding of the mechanism by which smoke stimulates germination in smoke responsive species and to devise new methods for eliciting a germination response in species which have not responded to current smoke procedures.

A study by Dixon *et al.* (1995) found that 23 Australian native species (15 families) showed a significant response to smoke stimulation. There remained a large group of species the seed of which showed low (<30%) or nil germination under glasshouse conditions even following the

application of smoke. Several of these species are known to germinate with the *in situ* application of smoke in native habitats. This suggests that some species require additional germination cues, acting with smoke to overcome seed dormancy.

Research conducted by S. Roche, presented in Chapter 2, examined the role of seed ageing in improving germination. A suite of 181 species with limited smoke response were treated with a number of commonly used seed pretreatments in combination with smoke.

An understanding of the way in which smoke overcomes dormancy in Australian native seed is required if smoke technology is to be effectively utilized. At present the mechanism of smoke stimulation of germination is still poorly understood. Chapter 3 covers an investigation into the mode of action of smoke in *Stylidium affine* (an indicator species) and *Actinotus leucocephalus*, a smoke responsive species with potential as a bedding plant.

Boronia megastigma is a species valued for its essential oils however its horticultural development has been hindered by difficulties in propagating it from seed. Chapter 4 involved a study which investigated ways of overcoming dormancy in this recalcitrant species.

1.5.2 Directive 2

The second directive focused on ways of improving the effectiveness of smoke application in order to make the technology more commercially available.

The three main methods of smoke application: aerosol smoking of punnets; aerosol smoking of seed; and aqueous smoke, have been refined and standardized for general horticultural use. A discussion of how these procedures have been refined (using indicator species) is outlined in Chapter 5.

The major limiting step in the development of smoke technology has been determining a simple way in which seed can be pretreated and primed with smoke and then stored for later use. Chapter 6 examines this issue using 4 important bedding species (*Conostylis candidans*, *C. setigera*, *Actinotus leucocephalus* and *Stylidium brunonianum*) and the indicator species, *Stylidium affine*.

The final chapter is a discussion covering and summarizing the completion of all the key objectives of this research programme as listed below (Table 1).

1.5.3 Key Objectives

1. Refine smoke stimulation of target species.
2. Devise methods for smoke stimulated germination of large seeded species, marginally germinable species and other species which have not responded to current smoke procedures.
3. Determine optimal smoke concentration of smoke fumigation exposure times for eliciting germination responses.
4. Refine smoke generation apparatus and methods for smoke application i.e. granulation, liquid application.
5. Develop smoke application procedures for stimulation of germination in field and evaluate the best methods for subsequent establishment of plants.
6. Develop procedures for direct application of smoke or the germination enhancing principle to seed i.e. coatings, pre-imbibing or encapsulating technology.
7. Develop a protocol for use of smoke germination technology in nursery propagation and possibly direct sowing of economically significant species.

SECTION 1.

REFINING SMOKE STIMULATION OF TARGET SPECIES AND OVERCOMING DORMANCY IN SPECIES INITIALLY UNRESPONSIVE TO SMOKE.

Research Team Members

S. Roche, A. Tieu, T.Vigilante & K.W. Dixon

CHAPTER 2. SEED AGEING AND SMOKE: PARTNER CUES IN THE AMELIORATION OF SEED DORMANCY IN SELECTED AUSTRALIAN NATIVE SPECIES

2.1 INTRODUCTION

The germination of Australian native seed is now known to be affected by a wide range of processes often acting synergistically (see Mott and Groves 1981; Bewley and Black 1985; Bell *et al.* 1993; Bell 1994a; Sukhvibul and Considine 1994; Plummer and Bell 1995). Smoke is recognized as an important germination cue for many species across a wide range of taxonomic groups. However, there is a dearth of information, and none in an Australian context, as to how smoke may interact with other dormancy-relieving processes in promoting germination (de Lange and Boucher 1993a, b; Pierce *et al.* 1995; van Staden *et al.* 1995c).

The present study follows the earlier investigation of Dixon *et al.* (1995) who found substantive heterogeneity in seed germination of Western Australian native species. It was reported that although the germination of a number of species could be positively influenced by the application of smoke or eluates of smoke, many species continued to display nil or low germination percentages under glasshouse or laboratory conditions. Further, several species (e.g. *Hibbertia* spp. and woody-fruited members of the Epacridaceae) which had responded in this minimal way, were observed to germinate in high numbers with the application of smoke to native habitats.

From a horticultural and rehabilitation perspective, maximum return for investment in seed acquisition is vital. Synchrony and speed of emergence as well as gross quantitative success continue to be of industrial as well as ecological interest. A number of plant species continue to be difficult or impossible to germinate and a variety of seed pretreatments has been employed toward this end (see Langkamp 1987; Williams *et al.* 1989; Paynter and Dixon 1991; Bell *et al.* 1993; Tan and Broadhurst 1993; Ashwath *et al.* 1994; Bell 1994a; Bunker 1994; Herpich *et al.* 1994; Jusaitis 1994; Loveys and Jusaitis 1994; Richmond and Ghisalberti 1994; Bell *et al.* 1995; Dixon *et al.* 1995; Osborne *et al.* 1995; Plummer *et al.* 1995; Schatral 1996).

The aims of the current study are to investigate (a) whether a decline in viability would take place as a result of seed ageing, (b) whether this supposed decline would affect the proportion of seed responding positively to smoke, (c) whether seed ageing prior to the application of smoke acts as a partner cue in the amelioration of obligate seed dormancy, as is regularly observed *in situ* with broad scale post-fire seed germination, and (d) if seed ageing alone would induce similar levels of germination to that induced by smoke application, as observed in nature with continuous recruitment regardless of the influences of fire.

2.2 METHODS

2.2.1 Seed Selection, Quality and Viability Decline

Seed of 181 species were selected on the basis of previously having shown low (< 30%) or nil germination under glasshouse conditions even following the application of smoke (see Dixon *et al.* 1995). A number of those selected are of interest to horticultural or rehabilitation professionals. For initial viability determinations random samples of 50-100 seeds were selected of each species and sectioned by hand to determine presence or absence of endosperm or embryo, and to check for any symptoms of decay which one would normally associate with inviable seed. Germination values were then expressed on the basis of proportions of the viable seed. In order to determine decline in viability after one year soil storage, three replicates ranging between 30 and 500 seed (depending on availability) were stored in punnets and watered throughout the first season (as for all control and smoke and, see below). At the commencement of the second season, seed was recovered, examined and sectioned as before.

2.2.2 Experiments

Seed numbers per replicate for most species ranged between 100 and 500 but, where seed of a species was limited, only 30 seeds per replicate were used. Seeds were sown in 14 x 8.5 cm plastic punnets containing a pasteurised soil mix of composted hardwood fines, hardwood sawdust and quartz sand in the ratio 1 : 3 : 2. No fertiliser was added. Seeds were spread evenly over the surface of the soil and covered with a layer of sieved quartz sand to a depth approximately equal to the diameter of the seed. Six replicates of seed were used for each

treatment except where otherwise stated. All punnets of sown seed were kept in a shaded glasshouse and watered as required. In order to approximate outdoor ambient conditions, regulation of temperature by thermostat-controlled cool ventilation was provided in summer and shading was removed from the glasshouse in winter.

Punnets were treated with aerosol smoke for 1 hour as described in section 1.3.2.

(a) Smoke responsiveness in fresh seed and method of application.

All 181 species were subjected to fresh seed trials, that is, seeds collected the previous year were sown and smoked as described above. Watering commenced in the autumn of 1994 (March) and concluded at the beginning of summer (November). This constituted the end of the first season.

To compare methods of smoke application, seed of 18 species were placed in petri dishes, subjected to 1h of smoke then kept in sealed containers for seven days prior to sowing. Control and smoked replicates were sown prior to smoke treatment where applicable.

Germinability was expressed as percentage of viable seed at the commencement of the experiment (initial viability).

(b) Smoke responsiveness in soil stored seed

Once scoring of the first season germination had concluded (November of the first year), all fresh seed punnets were allowed to dry out and remain undisturbed until autumn of the following year. Three control replicates remained unsmoked (thus becoming C/C in the second season), three control replicates were smoked for the first time (C/S) and three of the previously smoked punnets were smoked again (S/S). Watering and monitoring of germination recommenced in March of 1995. The second season was concluded at the beginning of November of that year. Germinability was expressed both as a percentage of the viable seed at the time of each germination trial, i.e. autumn 1994 (initial viability) and autumn 1995 (viability of one year old seed).

(c) Rapid ageing response compared to fresh seed

Fresh seed were represented by the Control and Smoked replicates (see a and b, above). Six rapid ageing methods were employed in the treatment of 32 species, but not all methods were trialed for each species (see Table 2.3).

Rapid ageing methods:

- ï Fruit removal (FR): any attached fruit or pericarp material was removed prior to sowing and smoke treatment. Soaking of seed for 24 h in water softened the material enough to be removed by hand.
- ï Nicking (NI): seeds were examined under a dissection microscope to determine the orientation of the embryo. Nicking took the form of a small piece of seed coat material being removed from the radicle end, if identifiable.
- ï Leaching (L): seeds were leached in ambient temperature running water for 3 days.
- ï Testa removal (TR) : the seed coat was removed under a dissection microscope.
- ï Acid (AC): seeds were plunged into concentrated H₂SO₄ followed by repeated rinsing in running water. The length of exposure to acid varied from one to 60 minutes and was determined by sampling seed at intervals and evaluating when/if significant damage had occurred to the endosperm (e.g. *Scaevola*). In the case of woody-fruited Epacridaceae, exposure times were determined when a > 45% survival of extracted embryos was obtained after 30 minutes to three hours immersion (S. Roche, unpublished data. For a detailed description of embryo extraction and germination methods see Meney and Dixon , 1995).
- ï Wet/dry cycles (WD): seeds were sown and punnets subjected to four WD cycles prior to smoke treatment where applicable. Each cycle consisted of watering punnets for 3 days and then drying out at 40°C for a further 3 days.

For all treatments, control replicates were unsmoked. Germinability was expressed as percentage of viable seed as determined at the commencement of the experiment (initial viability).

2.2.3 Statistical Analyses

Germination percentages were arcsine transformed prior to analysis (untransformed data appears in all tables and figures). Analyses of variance were used, except in those species subjected to soil storage as an ageing process. The same punnets were used in the second season and so repeated measures ANOVA were used to determine significance. However, while the samples used in these comparisons are not completely independent, the means themselves are, and so for clarity in presentation of such a large data set, results of the simple ANOVA appear in Table 2.2. Tukey's multiple comparison test was employed to separate means ($P < 0.05$) (using the Tukey-Kramer modification for unequal replicate frequencies where applicable, Day and Quinn 1989; Zar 1984). Analyses were performed using Abacus Concepts, Super ANOVA statistical package (1989).

2.3 RESULTS

2.3.1 Seed Quality and Viability Decline

Viability of fresh seed ranged between zero and 100%. Of 181 species, 50 had a viability of $< 40\%$, 24 had a viability of 40 - 60% and 107 had a viability of $> 60\%$. Of the 41 species failing to germinate in any treatment, 23 had an initial viability of $< 20\%$ (Table 2.1). These have been excluded from further comparative totals within this report.

For the species involved in soil storage trials, 88 out of 124 species (77%) had declined in viability by up to 50% from initial measurements, and 36 had declined by $> 50\%$ (Table 2.1). These declines in viability proved to have significant bearing on the results of the soil storage trials in at least half of the cases studied (see b, below).

Of the 140 species germinating under any treatment regime, 64 (46%) exhibited earlier (> 7 days) emergence with smoke, 42 (30%) exhibited more synchronous emergence and 24 (17%) exhibited both earlier and more synchronous germination patterns (Table 2.1).

2.3.2 Experiments

(a) Smoke responsiveness in fresh seed and method of application.

In 97 species (out of 140 species - 69%) germination of fresh seed was enhanced by the application of smoke after sowing. A further 20 (14%) showed improvement but not significantly so (Table 2.2). In *Bursaria spinosa*, *Drosera gigantea*, *Ptilotus polystachys* and *Schoenia cassiniana* germination was inhibited when seeds were exposed to 1h of smoke treatment (Table 2.2).

Eighteen species were subjected to direct smoking prior to sowing (see ds, Table 2.3). This treatment enhanced germination to an equal or greater extent than application of smoke after sowing in 11 of these 18 (e.g. *Isopogon ceratophyllous* and *Geleznovia verrucosa*, Table 2.3). *Bursaria spinosa*, *Caesia calliantha* and *Hovea chorizemifolia* showed a negative influence of direct application of smoke, the latter two being the more interesting in the sense that smoking the punnets *after* sowing induced a positive response over unsmoked controls.

Table 2.1 Viability of selected Australian native species. Fire response, renewal bud location, initial viability and viability after 1 year soil storage are indicated. S, seeder; R, resprouter; Ch, chamaephyte; Cr, cryptophyte; H, hemicryptophyte; G, geophyte; P, phanerophyte; T, therophyte. n.d., not determined.

Taxon	Fire response	Renewal bud location	Initial viability (%)	Viability after 1 year soil storage (%)
GYMNOSPERMAE				
Cupressaceae				
<i>Callitris intratropica</i> (F.Muell.) R.Baker	S	P	50	41
MONOCOTYLEDONAE				
Anthericaceae				
<i>Agrostocrinum scabrum</i> (R.Br.) Baillon	R	Cr	55	40
<i>Arthropodium strictum</i> Endl.	R	G	94	52
<i>Caesia calliantha</i> R.Br.	R	G	60	20
<i>Caesia parviflora</i> R.Br.	R	G	22	n.d.
<i>Chamaescilla corymbosa</i> (R.Br.) F.Muell.	R	G	93	12
<i>Johnsonia lupulina</i> R.Br.	R	Cr	85	38
<i>Laxmannia omnifertilis</i> Lindley	S	Ch	97	19
<i>Sowerbaea multicaulis</i> E.Pritzl	S	Ch	90	70
<i>Thysanotus multiflorus</i> R.Br.	S	Ch	93	74
<i>Tricoryne elatior</i> R.Br.	R	G	76	n.d.
Colchicaceae				
<i>Burchardia umbellata</i> R.Br.	R	G	93	28
Cyperaceae				
<i>Gahnia clarkei</i> Benth.	R	Cr	0	n.d.
<i>Gahnia lanigera</i> (R. Br.) Benth.	R	Cr	80	40
<i>Lepidosperma angustatum</i> R.Br.	R	Cr	7	n.d.
<i>Lepidosperma longitudinale</i> Labill.	R	Cr	0	n.d.
<i>Tetraria octandra</i> (Nees) Kuek.	R	Cr	0	n.d.
Dasyopogonaceae				
<i>Acanthocarpus preissii</i> Lehm.	R	Cr	100	65
<i>Dasyopogon bromeliifolius</i> R.Br.	R	Cr	0	n.d.
<i>Kingia australis</i> R.Br.	R	Ch	15	3
<i>Lomandra integra</i> T.D. MacFarlane	R	Cr	96	n.d.
<i>Lomandra micrantha</i> (Endl.) Ewart	R	Cr	96	n.d.
<i>Lomandra multiflora</i> R.Br.	R	Cr	96	n.d.
<i>Lomandra nigricans</i> T.D. MacFarlane	R	Cr	100	n.d.
<i>Lomandra preissii</i> (Endl.) Ewart	R	Cr	100	90
<i>Lomandra purpurea</i> (Endl.) Ewart	R	Cr	100	n.d.
<i>Lomandra sonderi</i> (F.Muell.) Ewart	R	Cr	90	n.d.
<i>Lomandra spartea</i> (Endl.) Ewart	R	Cr	100	n.d.
Haemodoraceae				
<i>Anigozanthos manglesii</i> D.Don	R	G	93	40
<i>Anigozanthos rufus</i> Labill.	R	G	100	40
<i>Blancoa canescens</i> Lindley	R	H	90	59
<i>Conostylis aculeata</i> R.Br.	R	H	97	20
<i>Haemodorum simplex</i> R.Br.	R	G	100	20
<i>Macropidia fuliginosa</i> (Hook.) Druce	R	H	49	32

Table 2.1 cont.

Taxon	Fire response	Renewal bud location	Initial viability (%)	Viability after 1 year soil storage (%)
Iridaceae				
<i>Orthrosanthus laxus</i> (Endl.) Benth.	R	Cr	93	34
<i>Patersonia glabrata</i> R.Br.	R	Cr	84	n.d.
<i>Patersonia occidentalis</i> R.Br.	R	Cr	67	58
<i>Patersonia</i> sp. aff. <i>fragilis</i>	R	Cr	86	32
<i>Patersonia umbrosa</i> Endl.	R	Cr	87	n.d.
Phormiaceae				
<i>Dianella amoena</i>	R	Cr	60	n.d.
G.W. Carr et P.F. Horsfall, <i>sp. nov.</i>				
<i>Dianella brevicaulis</i> (Ostenf.)	R	Cr	93	n.d.
G.W. Carr et P.F. Horsfall, <i>comb. et stat. nov.</i>				
<i>Dianella callicarpa</i>	R	Cr	64	n.d.
G.W. Carr et P.F. Horsfall, <i>sp. nov.</i>				
<i>Dianella</i> sp. aff. <i>longifolia</i>	R	Cr	80	n.d.
<i>Dianella revoluta</i> R.Br.	R	Cr	96	36
<i>Dianella revoluta</i> R.Br. var. <i>revoluta</i>	R	Cr	80	n.d.
<i>Dianella tarda</i>	R	Cr	88	n.d.
G.W. Carr et P.F. Horsfall, <i>sp. nov.</i>				
<i>Dianella tasmanica</i> J.D. Hook	R	Cr	50	n.d.
<i>Stypandra glauca</i> R.Br.	R	Cr	71	n.d.
Poaceae				
<i>Amphipogon amphipogonoides</i> (Steudel) Vick.	R?	?	33	15
<i>Tetrarrhaena laevis</i> R.Br.	R	?	30	n.d.
Restionaceae				
<i>Ecdeocolea monostachya</i> F.Muell.	R	Cr	63	n.d.
<i>Georgiella hexandra</i>	R	Cr	100	31
BG Briggs and LAS Johnson, in ed.				
DICOTYLEDONAE				
Amaranthaceae				
<i>Ptilotus auriculifolius</i> F.Muell.	S	T	19	n.d.
<i>Ptilotus axillaris</i> F.Muell.	S	T	6	n.d.
<i>Ptilotus drummondii</i> F.Muell.	S	T	4	n.d.
<i>Ptilotus exultatus</i> Nees	S	T	12	n.d.
<i>Ptilotus helipterioides</i> F.Muell.	S	T	10	n.d.
<i>Ptilotus nobilis</i> F.Muell.	S	T	4	n.d.
<i>Ptilotus polystachys</i> F.Muell.	S	T	43	n.d.
<i>Ptilotus rotindifolius</i> F.Muell.	S	T	20	n.d.
Apiaceae				
<i>Actinotus helianthi</i> Labill.	S	Ch	83	65
<i>Actinotus leucocephalus</i> Benth.	S	T	10	n.d.
<i>Pentapeletis peltigera</i> Hook.	R	H	17	n.d.
<i>Xanthosia huegelii</i> (Benth.) Steudel	R	H	67	38
Apocynaceae				
<i>Alyxia buxifolia</i> R.Br.	R	Ch	92	80
Asteraceae				

Table 2.1 cont.

Taxon	Fire response	Renewal bud location	Initial viability (%)	Viability after 1 year soil storage (%)
<i>Schoenia cassiniana</i> Steetz	S	T	80	n.d.
<i>Trichocline spathulata</i> J.H Wilis	R	G	3	n.d.
<i>Vittadinia gracilis</i> N.Burb.	S	T	30	n.d.
Chloanthaceae				
<i>Hemiphora elderi</i> F.Muell.	S	Ch	0	n.d.
<i>Lachnostachys eriobotrya</i> (F.Muel.) Druce	R	Ch	4	n.d.
<i>Lachnostachys verbascifolia</i> F.Muell	R	Ch	7	n.d.
<i>Pityrodia scabra</i> A.S. George	S	Ch	0	n.d.
Dilleniaceae				
<i>Hibbertia amplexicaulis</i> Steudel	R	Ch?	75	46
<i>Hibbertia commutata</i> Steudel	R	Ch?	97	67
<i>Hibbertia cunififormis</i> Labill.	R	Ch?	17	12
<i>Hibbertia lasiopus</i> Benth.	R?	Ch?	87	75
<i>Hibbertia mylnei</i> Benth.	R?	Ch?	21	16
<i>Hibbertia ovata</i> Steudel	R?	Ch?	71	43
<i>Hibbertia quadricolor</i> Domin, Vestn.	R?	Ch?	80	70
<i>Hibbertia riparia</i> Hoogl.	R?	Ch?	97	55
<i>Hibbertia sericea</i> Benth.	R?	Ch?	70	51
<i>Hibbertia serrata</i> Hotchk.	R	Ch?	46	18
Droseraceae				
<i>Drosera gigantea</i> Lindley	R	G	93	n.d.
<i>Drosera macrantha</i> ssp. <i>macrantha</i> Endl.	R	G	40	n.d.
<i>Drosera pallida</i> Lindley	R	G	83	n.d.
Epacridaceae				
<i>Acrotriche patula</i> R.Br.	R	Ch	80	50
<i>Andersonia involucrata</i> Sonder	S	Ch	96	38
<i>Andersonia latiflora</i> (F.Muell.) Benth.	S	Ch	97	27
<i>Astroloma foliosum</i> Sonder	R	Ch	74	59
<i>Astroloma pallidum</i> R.Br.	R	Ch	58	24
<i>Epacris obtusifolia</i> Smith	S	Ch	100	19
<i>Leucopogon capitellatus</i> DC.	R	Ch	73	36
<i>Leucopogon nutans</i> E. Pritzel	R	Ch	75	64
<i>Leucopogon propinquus</i> R.Br.	R	Ch	24	21
<i>Leucopogon verticillatus</i> R.Br.	R	Ch	25	6
<i>Styphelia tenuiflora</i> Lindley	R	Ch	28	25
Euphorbiaceae				
<i>Ricinocarpus glaucus</i> Endl.	S	Ch	100	50
Goodeniaceae				
<i>Brunonia australis</i> Smith	S	T	33	0
<i>Gonocarpus cordiger</i> (Fenzl) Endl.	S	?	5	n.d.
<i>Goodenia caerulea</i> R.Br	S	?	20	5
<i>Scaevola calliptera</i> Benth.	R?	?	60	26
<i>Scaevola crassifolia</i> Labill.	S	Ch	67	32
<i>Scaevola fasciculata</i> Benth.	R?	?	91	80

Scaevola pilosa Benth.

R?

?

33

8

Table 2.1 cont.

Taxon	Fire response	Renewal bud location	Initial viability (%)	Viability after 1 year soil storage (%)
<i>Velleia trinervis</i> Labill.	S	T	87	n.d.
Gyrostemonaceae				
<i>Tersonia cyathiflora</i> (Fenzl) A.S. George	S	T	67	41
Mimosaceae				
<i>Acacia cyclops</i> Cunn. ex Don	R	Ch	100	n.d.
Myoporaceae				
<i>Myoporum turbinatum</i> Chinnock	S	Ch	3	n.d.
Myrtaceae				
<i>Agonis linearifolia</i> (DC.) Schauer	R	Ch	80	5
<i>Astartea fascicularis</i> (Labill.) DC	R	Ch	47	14
<i>Baeckea camphorosmae</i> Endl.	R	Ch	52	30
<i>Calytrix breviseta</i> var. <i>breviseta</i> Lindley	S	Ch	63	18
<i>Calytrix depressa</i> (Turcz.) Benth.	R	Ch	54	20
<i>Calytrix flavescens</i> Cunn.	R	Ch	4	n.d.
<i>Calytrix fraserii</i> Cunn.	S	Ch	47	5
<i>Calytrix tetragona</i> Labill.	?	Ch	47	6
<i>Leptospermum spinescens</i> Endl.	R	Ch	90	23
<i>Sholtzia laxiflora</i> Benth.	R	Ch	0	n.d.
<i>Thryptomene saxicola</i> Schauer	?	Ch	10	n.d.
<i>Verticordia aurea</i> A.S. George	S	Ch	60	40
<i>Verticordia crisantha</i> Endl.	S	Ch	63	38
<i>Verticordia densiflora</i> Lindley	S	Ch	58	23
<i>Verticordia eriocephala</i> A.S. George	S	Ch	20	11
<i>Verticordia huegelii</i> Endl.	S	Ch	26	3
Papilionaceae				
<i>Hovea chorizemifolia</i> (Sweet) DC.	R	Ch	100	21
<i>Hovea pungens</i> Benth.	R	Ch	100	19
<i>Hovea trisperma</i> Benth.	R	Ch	100	10
Pittosporaceae				
<i>Billardiera varifolia</i> DC.	R	Ch	90	62
<i>Bursaria spinosa</i> Cav.	R	Ch	87	8
<i>Cheiranthra preissiana</i> Putterl.	R	Ch	97	5
<i>Sollya heterophylla</i> Lindley	R	Ch	100	n.d.
Polygalaceae				
<i>Comesperma virgatum</i> Labill.	R	Ch	87	14
Proteaceae				
<i>Conospermum incurvum</i> Lindley	S	Ch	66	55
<i>Conospermum triplinervium</i> R.Br.	S	Ch	70	60
<i>Grevillea polybotrya</i> Meissner	S	Ch	50	38
<i>Grevillea quercifolia</i> R.Br.	R	Ch	90	48
<i>Grevillea scapigera</i> A.S. George	S	Ch	90	80
<i>Grevillea wilsonii</i> Cunn.	R	Ch	93	27
<i>Hakea corymbosa</i> R.Br.	S	Ch	70	15
<i>Isopogon ceratophyllous</i> R.Br.	S	Ch	57	7

Table 2.1 cont.

Taxon	Fire response	Renewal bud location	Initial viability (%)	Viability after 1 year soil storage (%)
<i>Persoonia elliptica</i> R.Br.	R	Ch	86	45
<i>Persoonia longifolia</i> R.Br. (collected in field, > 1 year since seed fall)	R	Ch	55	34
<i>Persoonia longifolia</i> R.Br. (laboratory stored, < 1 year since collection)	R	Ch	80	58
<i>Persoonia saccata</i> R.Br.	R	Ch	100	43
<i>Petrophile drummondii</i> Meissner	S	Ch	23	7
<i>Petrophile linearis</i> R.Br.	R	Ch	6	0
<i>Stirlingia latifolia</i> (R.Br.) Steudel	R	Ch	30	22
Ranunculaceae				
<i>Clematis pubescens</i> Huegel ex Endl.	R	Ch	100	9
Rhamnaceae				
<i>Cryptandra arbutifolia</i> Fenzl	S	Ch	97	87
<i>Pomaderris obcordata</i> Fenzl	S	Ch	100	72
<i>Spyridium parvifolium</i> F.Muell. L.	S	Ch	100	86
Rutaceae				
<i>Boronia fastigiata</i> Bartling	R	Ch	80	19
<i>Boronia megastigma</i> Pl.Preiss	S	Ch	93	45
<i>Boronia spathulata</i> Lindley	R	Ch	77	18
<i>Boronia tenuis</i> (Lindley) Benth.	R	Ch	15	n.d.
<i>Boronia viminea</i> Lindley	R	Ch	73	20
<i>Correa reflexa</i> var. <i>cardinalis</i> (Labill.) Vent.	S?	Ch	83	n.d.
<i>Diplolaena dampieri</i> Desf.	S?	Ch	73	53
<i>Geleznowia verrucosa</i> Turcz.	S	Ch	83	43
Santalaceae				
<i>Exocarpus sparteus</i> R.Br.	S	Ch	77	27
<i>Leptomeria cunninghamii</i> Miq.	S	Ch	50	8
Solanaceae				
<i>Anthocercis littorea</i> Labill.	S	Ch	68	8
Stackhousiaceae				
<i>Stackhousia pubescens</i> A. Rich	R	H	60	53
<i>Tripterococcus brunonis</i> Endl.	R	H	78	54
Sterculiaceae				
<i>Lasiopetalum floribundum</i> Benth.	R	Ch	90	59
<i>Thomasia glutinosa</i> Lindley	R	Ch	93	34
Stylidiaceae				
<i>Stylidium affine</i> Sonder	S?	Ch	93	36
<i>Stylidium amoenum</i> R.Br.	R	Cr	90	40
<i>Stylidium brunonianum</i> Benth.	S	Ch	97	45
<i>Stylidium bulbiferum</i> Benth.	S	Ch	93	47
<i>Stylidium hispidum</i> Lindley	R	Cr	97	42
<i>Stylidium junceum</i> R.Br.	R	Cr	77	39
<i>Stylidium schoenoides</i> DC.	R	Cr	93	38
Thymelaeaceae				
<i>Pimelea ciliata</i> B.L. Rye	S	Ch	25	18

Table 2.1 cont.

Taxon	Fire response	Renewal bud location	Initial viability (%)	Viability after 1 year soil storage (%)
<i>Pimelea imbricata</i> R.Br.	S	Ch	27	16
<i>Pimelea leucantha</i> Diels	S	Ch	90	69
<i>Pimlea suaveolens</i> Meissner	S	Ch	15	3
Tremandraceae				
<i>Tetratheca hirsuta</i> Lindley	R	Ch	50	20
Violaceae				
<i>Hybanthus calycinus</i> F.Muell	R	Ch	55	38
<i>Hybanthus floribundus</i> (Lindley) F.Muell.	R	Ch	79	22

(b) Smoke responsiveness in soil stored seed

When evaluating soil storage as a method of seed ageing and hence as an agency in the breaking of dormancy, it became clear that it was necessary to calculate germination percentages based both upon the proportion of viable units at the commencement of the experiment, and at the conclusion of the first season. Both methods are represented in Table 2.2 for all species included in the soil storage experiments in order that direct comparisons can be made. Figure 2.1 is illustrative of the need for such a calibration.

For 11 of 110 species subjected to soil storage treatments (e.g. *Alyxia buxifolia* and *Thysanotus multiflorus*, Table 2.2), soil storage alone elicited a significantly improved germination response when compared to fresh seed (i.e. comparison of C with C/C). A further 17 species exhibited non-significant improvement (e.g. *Acanthocarpus preissii*, Table 2.2). Twenty-one species showed a significant decrease in germination in their second season (e.g. *Andersonia latiflora* and *Chamaescilla corymbosa*, Table 2.2)

Twenty five percent of species (e.g. *Conospermum incurvum* and *Patersonia occidentalis*, Table 2.2) required both soil storage and smoke for maximal germination response (i.e. comparison of S with C/S). However this percentage doubled when decline in viability was taken into account (55 species, e.g. *Baeckea camphorosmae* and *Orthrosanthus laxus*, Table 2.2).

Two thirds of the species tested (70/110) indicated that smoke plays a role in the optimum germination of seed after ageing in soil (i.e. comparisons between C/C, C/S and S/S), (e.g. *Acanthocarpus preissii* and *Stirlingia latifolia*, Table 2.2). Declines in seed viability of as little as 15% were implicated in the outcomes of analysis of smoke responsiveness in another six species (e.g. *Grevillea wilsonii*, Table 2.2).

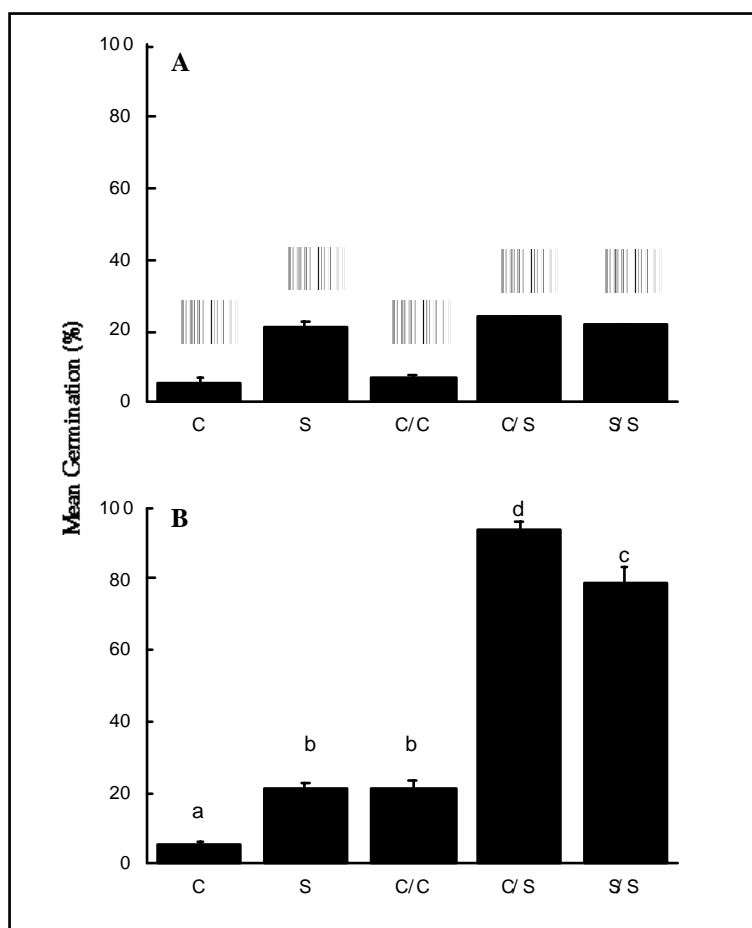


Fig. 2.1—Germination of *Orthrosanthus laxus* in response to smoke and soil storage. Data represent mean germination percentages of viable seed (\pm se); C, Control; S, Smoked; C/C, germination after one year storage; C/S, germination after one year storage (smoked prior to second season; S/S, germination after one year storage (smoked prior to both first and second season). Different letters indicate significant differences ($P < 0.05$) as determined by Tukey's multiple comparison (HSD). **A**, Germination percentages reflecting seed viability at commencement of experiment; **B**, Germination percentages reflecting seed viability after one year soil storage where applicable (C/C, C/S and S/S).

(c) Smoke responsiveness in rapidly aged seed

Of the 32 species subjected to rapid ageing regimes without smoke, four (*Acrotriche patula*, *Billardiera varifolia*, *Hibbertia commutata*, and *H. lasiopus*) exhibited significantly improved germination over fresh seed (i.e. a comparison of C versus FR C, TR C etc). This trend was observed in another two species (*Geleznovia verrucosa* and *Scaevola crassifolia*) but the improvement in germination of rapidly aged over fresh control replicates was not statistically significant (Table 2.3).

For 13 species (i.e. 40% of those treated, e.g. *Acrotriche patula*, *Billardiera varifolia*, *Hibbertia commutata* and *Scaevola fasciculata*, Table 2.3) application of smoke to rapidly aged seed either induced or increased germination above that of their unsmoked counterparts (i.e. a comparison of, for example, FR C with FR S). A further 5 species achieved non significant increases when rapidly aged seed was submitted to smoke (e.g. *Lomandra preissii*, Table 2.3).

As to the question of whether rapid ageing plus smoke would improve germination over smoke alone (i.e. a comparison of, for example, S with FR S), seven species (22%, e.g. *Alyxia buxifolia* and *Acrotriche patula*, Table 2.3) showed this to be the case, a further seven displayed a similar trend (e.g. *Hibbertia lasiopus*, S versus TR S, Table 2.3).

2.3.3 Absolute Requirement for Smoke and/or Seed Ageing

Twenty six of 140 species (19%) capable of germination under these conditions exhibited an absolute requirement for smoke (i.e. they did not germinate without smoke treatment) regardless of ageing regime (e.g. *Ricinocarpus glaucus* and *Verticordia* spp. Table 2.2).

Eleven of the 110 species (10%) subjected to ageing regimes showed an absolute requirement for ageing in seed germination, that is they did not germinate unless previously stored in soil. All 11 demonstrated the positive influence of smoke; 7 (e.g. *Johnsonia lupulina* and *Astroloma*) required both ageing and smoke in order to germinate. However, in all but one case (*Grevillea scapigera*) the germination achieved was < 15% (Table 2.2).

Some germination took place without ageing, but required smoke in 37 species. Germination in the first year (with smoke) was significantly lower than after soil storage (with or without smoke) in 16 (43%) of these 37 (e.g. *Acrotriche patula* and *Conospermum incurvum*, Table 2.2).

2.3.4 General Trends: Monocots, Dicots, Seeders and Resprouters

Initial viability of monocot species ($73 \pm 4\%$) was marginally higher than that of dicot species ($63 \pm 3\%$), as was that of resprouter species ($68 \pm 3\%$) over seeder species ($56 \pm 4\%$) (Fig. 2.2). Decline in viability after one year soil storage was steep in all groups (monocots $45 \pm 5\%$, dicots $36 \pm 3\%$, resprouters $40 \pm 3\%$, seeders $33 \pm 4\%$), however no significant differences between the monocots and dicots, or resprouters and seeders was evident (Fig. 2.2).

Germinability of smoke-treated viable seed increased after one year soil storage for all groups. Again, no significant differences were found between monocots and dicots, or resprouters and seeders (Fig. 2.2).

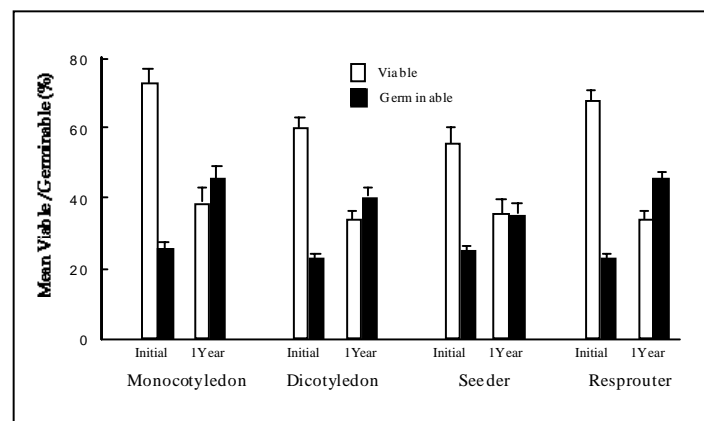


Fig. 2.2—Viability and germinability of monocot, dicot, seeder and resprouter species at commencement of experiment (initial) and after one year soil storage. Data represent mean percentages of viable seed (\pm se), and mean percent germination of viable seed (\pm se) when smoke treated (as this was the maximum for all groups).

The most conspicuous trend with respect to all groups (monocots, dicots, fire response, life form/renewal bud location) was that application of smoke significantly increased germination,

regardless of whether seed had or had not been previously soil stored (Fig. 2.3). Furthermore, even if these species were subjected to repeated treatment, germination percentages continued to be significantly higher than that of unsmoked controls (Fig. 2.3, compare C/C with S/S).

It must be noted however, that although the *percentage* of viable seed stimulated to germinate in the second season increased, the effect of a reduction in viability over the previous year was to achieve equivalent or fewer *numbers* of germinants after soil storage in all groups.

2.4 DISCUSSION

This publication presents the first reported response to smoke treatment for 75 species involved in this study. Where smoke responsiveness had been previously reported (Dixon *et al.* 1995) germination maxima were generally confirmed or exceeded. Smoke, especially when combined with other pre-treatments, continues to achieve germination percentages equivalent to or greater than treatments referred to in the literature prior to and since its discovery (for example Bell *et al.* 1993; Bell *et al.* 1995; Plummer *et al.* 1995; Schatral 1996).

In general, species germinated maximally either in their first year (fresh seed) or after soil storage. Rapid ageing was not as successful in eliciting a response, even when smoke was applied. Some exceptions to this rule were *Astroloma foliosum* (fruit removal before smoking), *Billardiera varifolia* (removal from pods before smoking), *Hibbertia commutata* (seed coat removal before smoking), *H. lasiopus* (seed coat removal before smoking), *H. sericea* (nicking before smoking), *Leucopogon capitellatus* (acid treatment before smoking), *Scaevola crassifolia* (acid treatment before smoking) and *S. fasciculata* (acid treatment before smoking).

Scarification with acids and other substances are common pre-treatments for seeds of many species (Sukhvibul and Considine 1994; Osborne *et al.* 1995). In preparation for the current

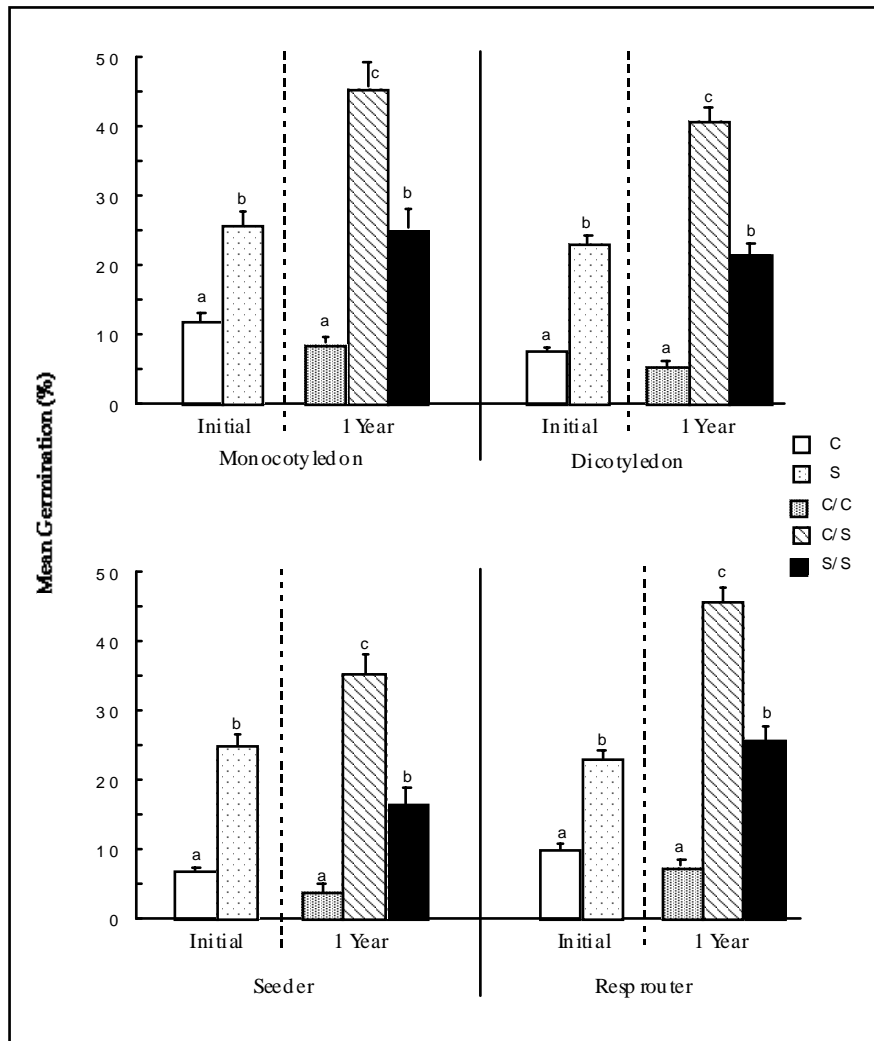


Fig. 2.3—Germination of monocot, dicot, seeder and resprouter species in response to smoke and soil storage. Data represent mean germination percentages of viable seed (\pm se) reflecting viability of seed at commencement of the experiment (initial) and after one year soil storage. C, Control; S, Smoked; C/C - second season - never smoked; C/S, smoked prior to second season only; S/S, smoked prior to both first and second season. Different letters indicate significant differences ($P < 0.05$) as determined by Tukey's multiple comparison (HSD).

study, over 50 species were plunged into concentrated H_2SO_4 in order to assess possible target species for investigation. Many of them exhibited similar traits to that of *Scaevola*, that is a spongy, or rigid-but-parchment-like seed coat becoming translucent or flexible in appearance after three to five minutes immersion in acid. Examination of the contents after treatment showed no signs of the characteristic inviable burnt appearance (S. Roche, pers. obs.). Given the dramatic improvement in germination, when combined with smoke, of

Scaevola fasciculata, this combination of treatments is certainly worthy of further investigation.

For *Hibbertia* species, neither testa removal, nicking, nor soil storage could be claimed as horticulturally practical treatments unless the species were rare or endangered. However, careful mechanical scarification at an intensity appropriate to damage the heavily cutinized testa (Schatral 1995) by a commercially available device prior to smoking, may prove to be a successful alternative. Unfortunately, the reason for woody-fruited Epacridaceae responding positively to various methods of smoke application *in situ* (Dixon *et al.* 1995; S. Roche unpublished data; P. Courtney and K. Meney unpublished data) but only minimally under horticultural conditions remains unexplained.

The finding that 61% of species tested were able to retain smoke responsiveness for at least seven days after direct treatment of the seed, augers well for the use of smoke in situations where tent smoking (see Dixon *et al.* 1995) is impractical or to be avoided for aesthetic reasons. Similarly, the seed could be pre-imbibed in dilute aqueous solutions of smoke and subsequently dehydrated, with retention of a positive response when imbibition was reinstated. In this study, *Hovea chorizemifolia* and *Caesia calliantha* showed a negative impact of direct application of smoke but a positive reaction when seed was sown before smoking. Similarly, *Drosera* species were negatively affected by smoking, but reports exist of an increase over unsmoked controls when the duration of treatment was 15 minutes or less (A. Lowrie, pers. comm.). For these, and possibly many other species, a shorter exposure time, or imbibition in dilute smoked water may be advantageous.

With the interest in bio-prospecting for Australian native flowers, seeds, fruit and phytochemicals gaining momentum, improving the commercial orcharding potential of species under consideration must be encouraged as a method through which the harvesting pressure on adult plants in the wild can ultimately be reduced. Relevant in this context is that vegetative propagation of many Australian plants can be equally as troublesome as propagation from seed. Smoke improves the germination response of a number of taxa and may therefore prove to have an important role to play in the reduction of habitat degradation.

The improvement in synchrony and/or speed of emergence with smoke treatment of fresh seed is interesting. The latter is possibly an important ecological consideration where rapid germination and establishment is advantageous, in particular where short-lived seasonal rainfall occurs in tandem with conducive ambient temperatures (Bell *et al.* 1993). It has been asserted that aqueous extracts of smoke stimulate root initiation and development in the hypocotyls of mung beans (Taylor and van Staden 1996). Increased vigour of smoke treated *Lysinema ciliatum* and *Sphenotoma capitatum* germinants was also noted by Roche (1993). If the biologically active compounds in smoke elicit the same response in a wide range of Australian native species, it might begin to explain both the relatively premature emergence of so many seedlings in this study (see Table 2.1), and the vigour of post-fire recruits observed *in situ*.

Processes involved in the physical degrading of, or the de-naturing of inhibitory chemicals within fruit material or seed coats is known to positively influence seed germination (van Staden and Brown 1973; de Lange and Boucher 1993a, b; Brits *et al.* 1995; Plummer *et al.* 1995; Richmond and Ghisalberti 1995; Schatral 1996). Seed burial has been implicated in this process (de Lange and Boucher 1993a, b; van Staden *et al.* 1994). Certainly the outcomes of this study reinforce these findings, they also highlight the extent to which transience in soil-borne seed banks may make many taxa vulnerable to repeated disturbance (see also Meney *et al.* 1994; Morgan 1995; Yates *et al.* 1995). Further, the results underline an important trade-off between viability decline and dormancy release which may influence a species' ability to persist under such deleterious pressure. For example, if it were necessary to estimate the viable and/or germinable seed bank of a smoke-responsive species such as *Orthrosanthus laxus* in a post-fire situation, given the results indicated in Figure 2.1A (C/S), one might suppose that 76% of seed shed in the year prior to the current season would remain ungerminated at the conclusion of the following winter, and could therefore contribute to further recruitment at a later stage. However, as Figure 2.1B shows, with a decline in viability in this species of 59% over one year, virtually all seed > 1 year old would have germinated in response to the fire. Any further recruitment could only come from seed shed in the season immediately preceding the fire but which had remained dormant, presumably due to a requirement for ageing (represented by δS_i in Fig. 2.1B).

Additionally, if recently-shed seed were not already buried, one might assume that the seed bank would be further depleted through combustion of unburied seeds or through predation (Yates *et al.* 1995). These findings, apparently elucidated for few Australian species, have far-reaching implications for responsible management of ecosystems, particularly with respect to fire as a management tool, and warrant further investigation as to seed viability decline both *ex* and *in situ*.

Such high levels of heterogeneity in Australian native seed germination both within and between species continues to be a fascinating conundrum. However from the results of this study, it appears that seed ageing and fire-related germination cues such as smoke may act as sequential partner cues in the amelioration of Australian native seed dormancy.

Table 2.2 The effects of smoke, soil storage and viability decline on the seed germination of selected Australian native species. The first group of numbers for each species represent mean percent germination (\pm se) of the viable seeds within test replicates at the commencement of the experiment; the group immediately below reflect the viability of seed after one year storage in soil where applicable (i.e. for C/C, C/S and S/S treatments). Means subtended by different letters were determined to be significantly different (* = $P < 0.05$; ** = $P < 0.005$; *** = $P < 0.0005$; n.s. = not significant) by Tukey's multiple comparison test (HSD). C, Control (unsmoked fresh seed); S, fresh seed smoked after sowing; C/C, after soil storage (never smoked); C/S, after soil storage (smoked prior to second season); S/S, after soil storage (smoked prior to both first and second season). Where a hyphen (-) appears, the species was not subjected to the treatment. Species which germinated greater than seven days earlier with smoke (E.G), and species which germinated more synchronously with smoke (S.G) are indicated by (■) and (i) respectively. Number of days to first emergence (Days) are also indicated.

Taxon	P value	Treatment					E.G	S.G	Days
		C	S	C/C	C/S	S/S			
GYMNOSPERMAE									
Cupressaceae									
<i>Callitris intratropica</i> (F. Muell.) R. Baker	***	1.3 (\pm 1.3) a	13.9 (\pm 1.8) b	0.0 a	0.0 a	0.0 a	■		32
MONOCOTYLEDONAE									
Anthericaceae									
<i>Agrostocrinum scabrum</i> (R.Br.)Baillon	***	0.0 a	0.5 (\pm 0.5) a	1.9 (\pm 0.9) a	6.7 (\pm 0.9) b	5.7 (\pm 1.6) b			58
	***	0.0 a	0.5 (\pm 0.5) a	4.9 (\pm 2.4) b	17.9 (\pm 2.7) c	15.3 (\pm 4.6) c			
<i>Arthropodium strictum</i> Endl.	***	29 (\pm 2.5) b	59.3 (\pm 3.2) c	10 (\pm 1.8) a	21.5 (\pm 1.5) b	6.8 (\pm 2.4) a			12
	***	29 (\pm 2.5) b	59.3 (\pm 3.2) c	21.5 (\pm 4.3) ab	53 (\pm 5) c	14.4 (\pm 5) a			
<i>Caesia calliantha</i> R.Br.	***	4 (\pm 0.9) b	10.9 (\pm 2) cd	0.0 a	4.7 (\pm 0.7) bc	11.7 (\pm 0.5) d			40
	***	4 (\pm 0.9) b	10.9 (\pm 2) c	0.0 a	24.8 (\pm 4) d	66 (\pm 3) e			
<i>Ceasia parviflora</i> R.Br.	n.s.	0	0	-	-	-			
<i>Chamaescilla corymbosa</i> (R. Br.) F. Muell.	***	47.9 (\pm 2.7) b	85.6 (\pm 4.9) c	0.8 (\pm 0.4) a	1.2 (\pm 0.6) a	0.4 (\pm 0.4) a			44
	***	47.9 (\pm 2.7) b	85.6 (\pm 4.9) c	6.7 (\pm 3.3) a	10 (\pm 6) a	3.3 (\pm 3.3) a			
<i>Johnsonia lupulina</i> R. Br.	**	0.0 a	0.0 a	0.0 a	8.1 (\pm 3) c	2.5 (\pm 0.5) b			38
	**	0.0 a	0.0 a	0.0 a	23.8 (\pm 9.7) a	6.8 (\pm 1.4) b			
<i>Laxmannia omnifertis</i> Lindley	***	22.1 (\pm 1.2) b	37.2 (\pm 1.4) c	0.2 (\pm 0.1) a	0.6 (\pm 0.2) a	1 (\pm 0.1) a		i	28
	***	22.1 (\pm 1.2) c	37.2 (\pm 1.4) d	0.9 (\pm 0.5) a	3.2 (\pm 0.9) b	5.1 (\pm 0.5) b			
<i>Sowerbaea multicaulis</i> E. Pritzel	***	0.0 a	1.4 (\pm 0.8) a	0.0 a	9.1 (\pm 2) b	10.2 (\pm 1.9) b			70
	***	0.0 a	1.4 (\pm 0.8) a	0.0 a	14.3 (\pm 3.4) b	16.3 (\pm 3.4) b			
<i>Thysanotus multiflorus</i> R. Br.	***	0.0 a	23.9 (\pm 2) d	6.4 (\pm 0.9) b	15.6 (\pm 1) c	9.7 (\pm 2.3) b	■	i	30
	***	0.0 a	23.9 (\pm 2) c	8.6 (\pm 1.2) b	21 (\pm 1.5) c	17.9 (\pm 5.5) c			
<i>Tricoryne elatior</i> R. Br.	n.s.	0	0	-	-	-			
Colchicaceae									
<i>Burchardia umbellata</i> R. Br.	***	24.1 (\pm 1.5) b	41.5 (\pm 1.2) c	2.4 (\pm 0.8) a	3.3 (\pm 1.1) a	-		i	34
	***	24.1 (\pm 1.5) b	41.5 (\pm 1.2) c	9 (\pm 3) a	12.4 (\pm 4.4) a	-			
Cyperaceae									
<i>Gahnia clarkei</i> Benth.	n.s.	0	0	-	-	-			
<i>Gahnia lanigera</i> (R. Br.) Benth.	***	0.6 (\pm 0.3) a	14.5 (\pm 1.9) c	0.8 (\pm 0.4) a	15.3 (\pm 2.8) c	6.1 (\pm 1) b	■		56
	***	0.6 (\pm 0.3) a	14.5 (\pm 1.9) b	2.1 (\pm 1) a	46.1 (\pm 10) c	16.2 (\pm 1.9) b			
<i>Lepidosperma angustatum</i> R. Br.	n.s.	0	0	-	-	-			
<i>Lepidosperma longitudinale</i> Labill.	n.s.	0	0	-	-	-			
<i>Tetragia octandra</i> (Nees) Kuek.	n.s.	0	0	-	-	-			

Table 2.2 cont.

Taxon	P value	C	S	C/C	C/S	S/S	E.G	S.G	Days
Dasypogonaceae									
<i>Acanthocarpus preissii</i> Lehm.	***	0.0 a	0.0 a	1.3 (±1.3) a	13.3 (±3.5) b	14.6 (±4.8) b			47
	***	0.0 a	0.0 a	2.1 (±0.9) a	24.3 (±2.1) b	27.7 (±2.4) b			
<i>Dasypogon bromeliifolius</i> R. Br.	n.s.	0	0	-	-	-			
<i>Kingia australis</i> R. Br.	n.s.	0	0	-	-	-			
<i>Lomandra integra</i> T.D. MacFarlane	n.s.	23.6 (±3.3)	23.6 (±6)	-	-	-	■		52
<i>Lomandra micrantha</i> (Endl.) Ewart	n.s.	49.3 (±3.5)	43.7 (±3.2)	-	-	-			44
<i>Lomandra multiflora</i> R. Br.	*	1.6 (±0.7) a	12.1 (±2.2) b	-	-	-	■		50
<i>Lomandra nigricans</i> T.D. MacFarlane	n.s.	16.4 (±1.5)	15.1 (±2.3)	-	-	-	■		77
<i>Lomandra preissii</i> (Endl.) Ewart	***	2.6 (±0.5) a	4.3 (±0.7) ab	7.8 (±1.6) b	22 (±2.2) d	14.2 (±1.4) c	■		62
	***	2.6 (±0.5) a	4.3 (±0.7) a	9.5 (±2.2) b	31.6 (±3.9) d	18.3 (±2.2) c			
<i>Lomandra purpurea</i> (Endl.) Ewart	n.s.	14.4 (±2.3)	21.8 (±3.2)	-	-	-	■		55
<i>Lomandra sonderi</i> (F. Muell.) Ewart	n.s.	0	1.3 (±0.8)	-	-	-			76
<i>Lomandra spartea</i> (Endl.) Ewart	n.s.	38.9 (±12)	44.5 (±14)	-	-	-	■		45
Haemodoraceae									
<i>Anigozanthos manglesii</i> D. Don	***	6.1 (±0.5) b	28.8 (±1.3) d	2.1 (±0.4) a	20.1 (2.3) c	4.2 (±0.7) ab		ï	23
	***	6.1 (±0.5) a	28.8 (±1.3) b	5.5 (±1.1) a	63.5 (±9.2) c	10.9 (±1.9) a			
<i>Anigozanthos rufus</i> Labill.	***	7.9 (±0.5) c	20.3 (±1.8) d	1.4 (±0.4) a	11.8 (0.5) c	4.4 (±0.3) b		ï	32
	***	7.9 (±0.5) b	20.3 (±1.8) c	3.6 (±1.1) a	33.6 (±1.6) d	11.5 (±1.2) b			
<i>Blancoa canescens</i> Lindley	***	0.0 a	47.7 (±10) c	0.0 a	28.2 (±4.5) bc	5.9 (±0.8) ab			42
	***	0.0 a	47.7 (±10) bc	0.0 a	68.2 (±12) c	10.7 (±1.4) ab			
<i>Conostylis aculeata</i> R. Br.	***	10.7 (±0.8) bc	30.9 (±2) d	2.1 (±0.4) a	13.2 (±0.7) c	8.1 (0.7) b	■	ï	25
	***	10.7 (±0.8) a	30.9 (±2) b	10.7 (±1.8) a	76.2 (±4.9) d	44 (±4.4) c			
<i>Haemodorum simplex</i> R. Br.	***	50.4 (±3) b	68.7 (±3) c	0.0 a	0.0 a	0.4 (±0.4) a	■		32
	***	50.4 (±3) b	68.7 (±3) c	0.0 a	0.0 a	2.1 (±2.1) a			
<i>Macropidia fuliginosa</i> (Hook.) Druce	**	0.0 a	0.0 a	2.6 (±2.6) a	15.4 (±4.4) b	-			55
	**	0.0 a	0.0 a	8.7 (±8.7) a	58.8 (±19) b				
Iridaceae									
<i>Orthrosanthus laxus</i> (Endl.) Benth.	**	5.7 (0.8) a	20.7 (±1.6) b	6.7 (±0.7) a	24.2 (±0.4) b	21.2 (±0.9) b	■	ï	33
	**	5.7 (0.8) a	20.7 (±1.6) b	21.1 (±2.3) b	94.1 (±1.8) d	79.3 (±4.3) c			
<i>Patersonia glabrata</i> R. Br.	**	0.0 a	5.2 (±1.3) b	5.2 (±3) b	43.1 (±4.5) c	63.8 (±10) d			38
<i>Patersonia occidentalis</i> R. Br.	***	0.4 (±0.2) a	12.4 (±1.3) bc	10.4 (±0.9) b	32.6 (±1.4) d	17 (±1.9) c	■	ï	45
	***	0.4 (±0.2) a	12.4 (±1.3) b	20 (±2.1) b	83.6 (±4.9) d	35.6 (±4.8) c			
<i>Patersonia</i> sp. aff. <i>fragilis</i>	**	11.8 (±2.3) ab	56.5 (±4.4) c	7.7 (±1.1) ab	17.9 (±2.7) b	5.7 (±1.1) a	■		44
	**	11.8 (±2.3) a	56.5 (±4.4) b	26.2 (±4) a	69.3 (±13) b	19.2 (±3.9) a			
<i>Patersonia umbrosa</i> Endl.	**	0.0 a	1.4 (±0.3) b	-	-	-			65
Phormiaceae									
<i>Dianella amoena</i> G.W. Carr et P.F. Horsfall, sp. nov.	n.s.	38.9 (±14)	44.4 (±14)	-	-	-			36

Table 2.2 cont.

Taxon	P value	C	S	C/C	C/S	S/S	E.G	S.G	Days
<i>Dianella brevicaulis</i> (Ostenf.) G.W. Carr et P.F. Horsfall, <i>comb.</i> <i>et stat. nov.</i>	**	0.0 a	35.7 (±5.4) b	-	-	-			36
<i>Dianella callicarpa</i> G.W. Carr et P.F. Horsfall, <i>sp. nov.</i>	n.s.	47.1 (±4.5)	52.4 (±9.4)	-	-	-			34
<i>Dianella</i> sp. aff. <i>longifolia</i>	**	33.3 (±2.4) a	59.7 (±3.7) b	-	-	-	■		34
<i>Dianella revoluta</i> R. Br.	***	6.3 (±0.9) a	34.7 (±3.5) c	8.3 (±2.3) a	20.8 (±2.4) b	5.6 (±1.4) a	■		38
	***	6.3 (±0.9) a	34.7 (±3.5) b	25.7 (±7.9) b	73.7 (±10) c	16.5 (±4.4) ab			
<i>Dianella revoluta</i> R.Br. var. <i>revoluta</i>	n.s.	0	0	-	-	-			
<i>Dianella tarda</i> G.W. Carr et P.F. Horsfall, <i>sp. nov.</i>	*	21.4 (±4.3) a	53.7 (±9) b	-	-	-			34
<i>Dianella tasmanica</i> J. D. Hook	n.s.	2.2 (±2.2)	4.4 (±2.3)	-	-	-			
<i>Stypandra glauca</i> R. Br.	n.s.	0	0	-	-	-			
Poaceae									
<i>Amphipogon amphipogonoides</i> (Steudel) Vick.	***	19 (±1.5) c	53.2 (±4.5) d	2.7 (±0.8) a	9.7 (±0.8) bc	6.7 (±0.8) ab		ï	16
	***	19 (±1.5) a	53.2 (±4.5) bc	18.4 (±6) a	71.5 (±7) c	47.7 (±7) b			
<i>Tetrarhaena laevis</i> R. Br.	**	1.3 (±1.3) a	17.7 (±2.6) b	-	-	-	■	ï	23
Restionaceae									
<i>Ecdeocolea monostachya</i> F. Muell.	n.s.	0	0	-	-	-			
<i>Georgiella hexandra</i> BG Briggs and LAS Johnson, in ed.	***	0.0 a	6.4 (±0.7) b	0.0 a	21.1 (±1.8) c	8.1 (±1.9) b			42
	***	0.0 a	6.4 (±0.7) b	0.0 a	86.7 (±9.2) d	28.6 (±7.4) c			
DICOTYLEDONAE									
Amaranthaceae									
<i>Ptilotus auriculifolius</i> F. Muell.	n.s.	0	0	0	0	0			
<i>Ptilotus axillaris</i> F. Muell.	n.s.	0	0	-	-	-			
<i>Ptilotus drummondii</i> F. Muell.	n.s.	0	0	-	-	-			
<i>Ptilotus exultatus</i> Nees	n.s.	0	0	-	-	-			
<i>Ptilotus helipteroides</i> F. Muell.	n.s.	0	0	-	-	-			
<i>Ptilotus nobilis</i> F. Muell.	n.s.	0	0	-	-	-			
<i>Ptilotus polystachys</i> F. Muell.	*	21 (±0.6) b	14.3 (±1.7) a	-	-	-			14
<i>Ptilotus rotundifolius</i> F. Muell.	n.s.	0	0	-	-	-			
Apiaceae									
<i>Actinotus helianthi</i> Labill.	**	15 (±9) b	56.2 (±3.4) d	7.8 (±0.5) a	29.9 (±2) c	7.4 (1.2) a		ï	30
	**	15 (±9) a	56.2 (±3.4) b	13.1 (±0.8) a	66 (±6) b	12.3 (±2.2) a			
<i>Actinotus leucocephalus</i> Benth.	n.s.	0	0	-	-	-			
<i>Pentapeletis peltigera</i> Hook.	n.s.	0	0	-	-	-			
<i>Xanthosia huegelii</i> (Benth.) Steudel	***	0.0 a	13.9 (±1.9) c	1.7 (±0.8) a	13.9 (±2.2) c	5.7 (±0.8) b	■		44
	***	0.0 a	13.9 (±1.9) c	4.3 (±2.1) b	36.7 (±5.7) d	18 (±2.7) c			
Apocynaceae									
<i>Alyxia buxifolia</i> R. Br.	***	0.0 a	2.6 (±1.2) a	18.6 (±1.7) b	34.2 (±3.5) c	24.8 (±1.8) bc	■	ï	35
	***	0.0 a	2.6 (±1.2) a	28.7 (±3.4) b	66 (±8) c	41.5 (±4) b			
Asteraceae									
<i>Myriocephalus stuartii</i> Benth.	**	0.0 a	0.0 a	1.3 (±0.3) c	1.8 (±0.3) c	0.5 (±0.3) b			28
	**	0.0 a	0.0 a	3.1 (±0.6) c	4.3 (±0.6) c	1.2 (±0.6) b			

Table 2.2 cont.

Taxon	P value	C	S	C/C	C/S	S/S	E.G	S.G	Days
<i>Schoenia cassiniana</i> Steetz	**	32.6 (±3.2) b	6.6 (±1.6) a	-	-	-			10
<i>Trichocline spathulata</i> J.H Wiis	n.s.	14.7 (±4.6)	29.5 (±8.3)	-	-	-		ï	27
<i>Vittadinia gracilis</i> N. Burb.	n.s.	0	0	-	-	-			
Chloanthaceae									
<i>Hemiphora elderi</i> F. Muell.	n.s.	0	0	-	-	-			
<i>Lachnostachys eriobotrya</i> (F. Muel.) Druce	n.s.	0	0	-	-	-			
<i>Lachnostachys verbascifolia</i> F. Muell	n.s.	0	0	-	-	-			
<i>Pityrodia scabra</i> A.S. George	n.s.	0	0	-	-	-			
Dilleniaceae									
<i>Hibbertia amplexicaulis</i> Steudel	***	0.0 a	3.1 (±1.1) ab	2.5 (±1.2) ab	14.8 (±3.7) c	7.4 (±2.1) c	■		73
	***	0.0 a	3.1 (±1.1) a	5.6 (±2.8) ab	38.8 (±11) c	17.7 (±5.4) b			
<i>Hibbertia commutata</i> Steudel	***	0.0 a	2.4 (±0.9) ab	2.8 (±0.7) b	33.3 (±4.3) c	29.2 (±2.4) c	■		58
	***	0.0 a	2.4 (±0.9) a	4.3 (±1.1) a	76.5 (±14) b	61.9 (±7) b			
<i>Hibbertia lasiopus</i> Benth.	***	0.3 (±0.3) a	11.8 (±1.2) b	0.0 a	29 (±1.6) c	12.4 (±3.1) b	■		70
	***	0.3 (±0.3) a	11.8 (±1.2) b	0.0 a	54.7 (±4.2) c	19.2 (±5.5) b			
<i>Hibbertia ovata</i> Steudel	***	0.5 (±0.5) a	6.1 (±1) b	6.7 (±1.9) b	19.9 (±3.3) c	22.2 (±2.3) c			47
	***	0.5 (±0.5) a	6.1 (±1) ab	16.8 (±5) b	59 (±11) c	66 (±8.8) c			
<i>Hibbertia quadricolor</i> Domin, Vestn.	***	0.0 a	5.7 (±1.3) b	2.5 (±1.2) ab	26.9 (±5.8) c	23.1 (±4.4) c	■		54
	***	0.0 a	5.7 (±1.3) a	3.8 (±1.9) a	55.4 (±17) b	44 (±11) b			
<i>Hibbertia riparia</i> Hoogl.	***	1.1 (±0.3) a	17.1 (±0.9) b	1.7 (±0.5) a	33.6 (±0.9) c	13.1 (±0.9) b	■	ï	44
	***	1.1 (±0.3) a	17.1 (±0.9) b	3.1 (±0.9) a	92.2 (±4) d	27.4 (±2.4) c			
<i>Hibbertia sericea</i> Benth.	***	0.0 a	14 (±4.2) bc	9.3 (±1.3) b	26.6 (±4.3) c	26.6 (±2.3) c	■		47
	***	0.0 a	14 (±4.2) b	20.3 (±3.2) b	73.9 (±11) c	71.5 (±5) c			
<i>Hibbertia serrata</i> Hotchk.	*	0.0 a	1.9 (±0.6) b	0.0 a	1.9 (±0.9) b	0.0 a	■		40
	*	0.0 a	1.9 (±0.6) a	0.0 a	10.8 (±5.4) b	0.0 a			
Droseraceae									
<i>Drosera gigantea</i> Lindley	**	18.1 (±1.4) b	9.4 (±1.5) a	-	-	-			49
<i>Drosera macrantha</i> ssp. <i>macrantha</i> Endl.	n.s.	2.1 (±0.9)	1.3 (±0.6)	-	-	-			53
<i>Drosera pallida</i> Lindley	n.s.	14.1 (±4.2)	6.4 (±2.4)	-	-	-			57
Epacridaceae									
<i>Acrotriche patula</i> R.Br.	***	0.0 a	0.7 (±0.3) b	16.7 (±0.5) c	26.7 (±1.9) d	24.7 (1.6) d		ï	64
	***	0.0 a	0.7 (±0.3) a	40 (±1.4) b	73.1 (±7) c	65.9 (±6) c			
<i>Andersonia involucrata</i> Sonder	***	5.2 (±0.9) b	20.9 (±1.8) c	0.6 (±0.1) a	7.8 (±0.6) b	0.6 (±0.1) a	■	ï	34
	***	5.2 (±0.9) b	20.9 (±1.8) c	1.6 (±0.2) a	22.5 (±1.8) c	1.6 (±0.3) a			
<i>Andersonia latiflora</i> (F. Muell.) Benth.	***	19.9 (±2.7) b	55.3 (±5.5) c	0.6 (±0.1) a	6.2 (±0.5) a	4.6 (±0.2) a		ï	32
	***	19.9 (±2.7) b	55.3 (±5.5) c	2.1 (±0.5) a	24.7 (±2.3) b	1.7 (±0.5) a			
<i>Astroloma foliosum</i> Sonder	**	0.0 a	0.0 a	0.0 a	5.4 (±1.5) b	3.6 (±0.9) b			39
	**	0.0 a	0.0 a	0.0 a	9.8 (±2.9) b	6.3 (±1.7) b			
<i>Astroloma pallidum</i> R.Br.	**	0.0 a	0.0 a	0.0 a	5.6 (±1.1) b	5.5 (±2.2) b			45
	**	0.0 a	0.0 a	0.0 a	21.9 (±4.5) b	22.3 (±9.4) b			
<i>Epacris obtusifolia</i> Smith	***	7.8 (±1.9) c	20.8 (±2.5) d	0.8 (±0.3) ab	2.6 (±0.2) bc	0.0 a	■	ï	23
	***	7.8 (±1.9) bc	20.8 (±2.5) d	4.4 (±1.7) b	13.8 (±1) cd	0.0 a			

Table 2.2 cont.

Taxon	P value	C	S	C/C	C/S	S/S	E.G	S.G	Days
<i>Leucopogon capitellatus</i> DC.	***	0.0 a	19.4 (±2.5) b	2.4 (±1.3) a	19 (±3.6) b	14.2 (±2.7) b			56
	***	0.0 a	19.4 (±2.5) b	6.9 (±4) ab	66.7 (±14) c	46.9 (±10) c			
<i>Leucopogon nutans</i> E. Pritzel	***	0.0 a	9.5 (±3) bc	2.3 (±2.3) ab	19 (±2.4) c	21.4 (±7.1) c			80
	***	0.0 a	9.5 (±3) a	4 (±4) a	37.1 (±5.5) b	46.2 (±19) b			
<i>Leucopogon propinquus</i> R. Br.	***	0.0 a	0.0 a	0.0 a	11.1 (±2.8) b	11.1 (±2.8) b			60
	***	0.0 a	0.0 a	0.0 a	60.6 (±17) b	60.6 (±17) b			
<i>Leucopogon verticillatus</i> R. Br.	n.s.	0	0	0	0	0			
<i>Styphelia tenuiflora</i> Lindley	n.s.	0	0	0	0	0			
Euphorbiaceae									
<i>Ricinocarpus glaucus</i> Endl.	***	0.0 a	20 (±3.6) b	0.0 a	16.7 (±3.3) b	13.3 (±3.3) b			36
	**	0.0 a	20 (±3.6) b	0.0 a	40.7 (±9.2) c	31.5 (±9.2) bc			
Goodeniaceae									
<i>Brunonia australis</i> Smith	***	4.1 (±1.2) b	20.2 (±1.8) c	0.0 a	0.0 a	0.0 a	■		20
<i>Gonocarpus cordiger</i> (Fenzl) Endl.	n.s.	2.8 (±0.8)	5.2 (±1)	-	-	-			45
<i>Goodenia caerulea</i> R.Br	n.s.	0	0	0	0	0			
<i>Scaevola calliptera</i> Benth.	***	0.0 a	13.8 (±2.4) b	1.2 (±1.2) a	13.1 (±3.1) b	8.3 (±1.2) b	■	ï	21
	***	0.0 a	13.8 (±2.4) bc	4.7 (±4.7) ab	59.1 (±15.7) d	35.1 (±5.5) cd			
<i>Scaevola crassifolia</i> Labill.	***	33.8 (±2.4) b	47.3 (±2.6) c	10.2 (±1.5) a	10.8 (±3) a	15 (±0.4) a		ï	25
	*	33.8 (±2.4) a	47.3 (±2.6) bc	35.7 (±6.2) ab	38.7 (±12.) abc	55.2 (±1.6) c			
<i>Scaevola fasciculata</i> Benth.	***	0.0 a	3.3 (±0.3) c	0.6 (±0.3) b	73.8 (±1.9) e	54.5 (±2.3) d	■	ï	31
	***	0.0 a	3.3 (±0.3) b	0.8 (±0.4) a	92.5 (±2.5) d	70.4 (±3.1) c			
<i>Scaevola pilosa</i> Benth.	n.s.	0	0	0	0.6 (±0.6)	0.6 (±0.6)			
<i>Velleia trinervis</i> Labill.	*	61.3 (±6.3) a	82.2 (±2.3) b	-	-	-			15
Gyrostemonaceae									
<i>Tersonia cyathiflora</i> (Fenzl) A.S. George	***	0.0 a	3.5 (±0.7) b	0.0 a	7 (±1.3) c	2 (±0.5) b			22
	***	0.0 a	3.5 (±0.7) b	0.0 a	17.1 (±3.2) c	5.1 (±1.3) b			
Mimosaceae									
<i>Acacia cyclops</i> Cunn. ex Don	*	38.2 (±.7) a	45 (±2.1) b	-	-	-	■	ï	15
Myoporaceae									
<i>Myoporum turbinatum</i> Chinnock	n.s.	0	0	-	-	-			
Myrtaceae									
<i>Agonis linearifolia</i> (DC.) Schauer	***	7.9 (±1.1) b	24.4 (±1.1) c	0.0 a	0.0 a	0.0 a		ï	23
<i>Astartea fascicularis</i> (Labill.) DC	***	6 (±1.1) c	26.4 (±1.7) d	0.0 a	1.5 (±0.3) b	0.0 a			40
	***	6 (±1.1) b	26.4 (±1.7) d	0.0 a	11.2 (±2.3) c	0.0 a			
<i>Baeckea camphorosmae</i> Endl.	***	1.7 (±0.3) ab	13.2 (±0.7) c	0.5 (±0.1) a	10.4 (±1.8) c	1.9 (±0.3) b			60
	***	1.7 (±0.3) a	13.2 (±0.7) c	1.5 (±0.4) a	38.9 (±7.8) d	6.7 (±1.1) b			
<i>Calytrix breviseta</i> var. <i>breviseta</i> Lindley	***	7 (±1.5) b	20.5 (±1.4) c	0.0 a	7.6 (±1) b	-	■		31
	***	7 (±1.5) b	20.5 (±1.4) c	0.0 a	45.4 (±7) d	-			
<i>Calytrix depressa</i> (Turcz.) Benth.	***	41.2 (±3.8) c	71.3 (±5.6) d	0.0 a	8 (0.8) b	0.0 a	■		40
	***	41.2 (±3.8) b	71.3 (±5.6) c	0.0 a	43.6 (±4.8) b	0.0 a			
<i>Calytrix flavescens</i> Cunn.	n.s.	0	0	-	-	-			

Table 2.2 cont.

Taxon	P value	C	S	C/C	C/S	S/S	E.G	S.G	Days
<i>Calytrix fraserii</i> Cunn.	***	4.4 (±0.9) b	12.7 (±1.6) c	0.0 a	1.3 (±0.6) ab	0.6 (±0.6) a	■	ï	32
	**	4.4 (±0.9) ab	12.7 (±1.6) ab	0.0 a	25.8 (±12) b	12.9 (±12) ab			
<i>Calytrix tetragona</i> Labill.	***	12.8 (±1) c	25.7 (±2.5) d	0.5 (±0.5) a	2.8 (±0.6) b	0.0 a	■		39
	***	12.8 (±1) bc	25.7 (±2.5) cd	9.7 (±9.7) ab	49.2 (±9) d	0.0 a			
<i>Leptospermum spinescens</i> Endl.	***	39.2 (±4.7) a	88.3 (±2.8) b	-	-	-	■	ï	31
<i>Sholtzia laxiflora</i> Benth.	n.s.	0	0	-	-	-			
<i>Thryptomene saxicola</i> Schauer	n.s.	0	0	-	-	-			
<i>Verticordia aurea</i> A.S. George	***	0.0 a	16.7 (±1) c	0.0 a	18.7 (±3) c	6.3 (±1.3) b			60
	*	0.0 a	16.7 (±1) b	0.0 a	77.8 (±12) c	31.7 (±6) b			
<i>Verticordia chrysantha</i> Endl.	***	0.0 a	42.8 (±3.8) c	0.0 a	33.3 (±3) c	4.6 (±0.6) b			31
	***	0.0 a	42.8 (±3.8) c	0.0 a	87.7 (±7.9) d	20 (±1.5) b			
<i>Verticordia densiflora</i> Lindley	***	0.0 a	28.1 (±1.8) c	0.0 a	21.1 (±0.9) b	0.7 (±0.7) a			44
	***	0.0 a	28.1 (±1.8) b	0.0 a	91.7 (±3.9) c	4 (±4) a			
<i>Verticordia eriocephala</i> A.S. George	***	0.0 a	35.5 (±2) c	0.0 a	7.8 (±1.3) b	0.0 a			47
	***	0.0 a	35.5 (±2) b	0.0 a	71.6 (±12) c	0.0 a			
<i>Verticordia huegellii</i> Endl.	**	0.0 a	26.2 (±2.4) b	0.0 a	0.0 a	0.0 a			62
Papilionaceae									
<i>Hovea chorizemifolia</i> (Sweet) DC.	***	51 (±3.7) b	66.6 (±2.2) c	-	-	-			36
<i>Hovea pungens</i> Benth.	n.s.	6.9 (±1.4)	11.7 (±1.6)	-	-	-	■		22
<i>Hovea trisperma</i> Benth.	***	11.4 (±1.3) a	34.6 (±2.8) b	-	-	-	■		24
Pittosporaceae									
<i>Billardiera varifolia</i> DC.	***	0.0 a	0.9 (±0.5) a	6.7 (±1.1) b	26.7 (±2.3) c	20 (±5.2) c	■	ï	48
	***	0.0 a	0.9 (±0.5) a	11.6 (±2.1) b	59 (±7) c	42 (±13) c			
<i>Bursaria spinosa</i> Cav.	***	31.8 (±1.5) c	16.2 (±1.1) b	-	-	-			18
<i>Cheiranthra preissiana</i> Putterl.	***	61.8 (±3.3) a	91.8 (±2.6) b	-	-	-	■	ï	22
<i>Sollya heterophylla</i> Lindley	***	36.1 (±2.5) a	70.8 (±4.5) b	-	-	-		ï	34
Polygalaceae									
<i>Comesperma virgatum</i> Labill.	***	11.7 (±2) b	30.7 (±4.3) c	0.8 (±0.8) a	7.5 (±1.4) b	0.0 a			46
	***	11.7 (±2) bc	30.7 (±4.3) c	6 (±6) ab	58.6 (±9) d	0.0 a			
Proteaceae									
<i>Conospermum incurvum</i> Lindley	***	0.0 a	5.3 (±0.8) b	3.5 (±2.2) b	25.7 (±0.9) c	32.3 (±1.3) c			28
	***	0.0 a	5.3 (±0.8) b	6.8 (±4.3) b	63.2 (±2.8) c	87 (±5.3) d			
<i>Conospermum triplinervium</i> R. Br.	***	0.0 a	2.9 (±0.6) b	1.9 (±1.2) ab	22.4 (±3.4) c	25.2 (±2.5) c			23
	***	0.0 a	2.9 (±0.6) a	3.3 (±2.2) a	48.9 (±9.2) b	58.1 (±14) b			
<i>Grevillea polybotrya</i> Meissner	***	7.2 (±1.3) a	18.8 (±1.7) b	3.6 (±2.1) a	27.9 (±2.4) b	6 (±2.4) a	■		26
	***	7.2 (±1.3) a	18.8 (±1.7) a	7.6 (±4.4) a	76.5 (±9.4) b	12.9 (±5.5) a			
<i>Grevillea quercifolia</i> R.Br.	***	1.6 (±1) a	19.8 (±1.5) c	9.5 (±3.7) abc	15.9 (±4.2) bc	6.3 (±3.2) ab	■		25
	**	1.6 (±1) a	19.8 (±1.5) abc	31 (±17) bc	54 (±17) c	19.4 (±9.7) ab			
<i>Grevillea scapigera</i> A.S. George	***	0.0 a	0.0 a	0.0 a	20 (±10) b	50 (±25) b			46
<i>Grevillea wilsonii</i> Cunn.	***	4.5 (±1.6) a	34.8 (±5.1) b	1.8 (±1.8) a	12.5 (±4.7) a	7.2 (±1.7) a	■		34
	***	4.5 (±1.6) a	34.8 (±5.1) ab	7 (±7) a	55.2 (±23) b	28.8 (±7.8) ab			

Table 2.2 cont.

Taxon	P value	C	S	C/C	C/S	S/S	E.G	S.G	Days
<i>Hakea corymbosa</i> R. Br.	***	19.5 (±3) b	75.7 (±6) c	0.0 a	4.3 (±4.3) a	0.0 a	■		23
	***	19.5 (±3) b	75.7 (±6) c	0.0 a	17.3 (±17) a	0.0 a			
<i>Isopogon ceratophyllous</i> R. Br.		0.0 a	22.2 (±5.1) b	-	-	-			36
<i>Persoonia elliptica</i> R. Br.	n.s.	0	0	0	0	0			
<i>Persoonia longifolia</i> R.Br. (collected in field, > 1 year since seed fall)	n.s.	0	0	3 (±3)	6.1 (±3)	6.1 (±6.1)			70
<i>Persoonia longifolia</i> R.Br. (laboratory stored, < 1 year since collection)	**	0.0 a	0.0 a	0.0 a	0.0 a	6.1 (±3) b			78
<i>Persoonia saccata</i> R. Br.	**	0.0 a	0.0 a	0.0 a	2.4 (±2.4) ab	5.5 (±2.7) b			49
<i>Petrophile drummondii</i> Meissner	***	22.5 (±2.2) b	62.5 (±5.2) c	0.0 a	0.0 a	0.0 a	■	ï	23
<i>Petrophile linearis</i> R. Br.	n.s.	18.2 (±9)	36.4 (±9)	-	-	-			25
<i>Stirlingia latifolia</i> (R. Br.) Steudel	***	0.0 a	6.5 (±0.5) b	0.3 (±0.3) a	24.7 (±3.7) c	1.1 (±1.1) a	■		48
	***	0.0 a	6.5 (±0.5) a	0.9 (±0.9) a	82.4 (±12) b	3.9 (±3.9) a			
Ranunculaceae									
<i>Clematis pubescens</i> Huegel ex Endl.	***	0.0 a	22.7 (±1.7) b	0.0 a	0.9 (±0.5) a	0.0 a			23
	***	0.0 a	22.7 (±1.7) c	0.0 a	9.7 (±5.6) b	0.0 a			
Rhamnaceae									
<i>Cryptandra arbutifolia</i> Fenzl	**	1.2 (±0.2) bc	1.7 (±0.1) c	0.1 (±0.1) a	0.4 (±0.1) b	0.4 (±0.1) b			23
	**	1.2 (±0.2) bc	1.7 (±0.1) c	0.2 (±0.2) a	0.5 (±0.1) b	0.5 (±0.1) b			
<i>Pomaderris obcordata</i> Fenzl	**	0.4 (±0.1) a	0.5 (±0.1) a	0.2 (±0.2) a	1 (±0.2) ab	2.4 (±0.3) b	■		48
	***	0.4 (±0.1) ab	0.5 (±0.1) ab	0.3 (±0.3) a	1.4 (±0.3) bc	3.3 (±0.5) c			
<i>Spyridium parvifolium</i> F.Muell. L.	**	0.2 (±0.1) a	1.2 (±0.2) b	0.0 a	0.0 a	0.0 a	■		38
Rutaceae									
<i>Boronia fastigiata</i> Bartling	n.s.	0	0.6 (±0.3)	0	2.3 (±1.1)	1.7 (±0.9)			34
	*	0.0 a	0.6 (±0.3) ab	0.0 a	12.3 (±6.1) b	9.1 (±5.3) b			
<i>Boronia megastigma</i> Pl. Preiss	**	0.0 a	0.0 a	1.1 (±0.6) ab	5.2 (±0.7) c	2.2 (±1.7) b			46
	**	0.0 a	0.0 a	2.5 (±1.4) ab	12.2 (±1.8) c	5.2 (±4) b			
<i>Boronia spathulata</i> Lindley	n.s.	0	0	0	0	0			
<i>Boronia tenuis</i> (Lindley) Benth.	**	3.2 (±3.2) a	57.1 (±16) b	-	-	-	■		40
<i>Boronia viminea</i> Lindley	**	1.1 (±0.2) ab	4.4 (±1.2) c	0.0 a	2.2 (±0.3) bc	0.7 (±0.3) ab	■		38
	**	1.1 (±0.2) ab	4.4 (±1.2) b	0.0 a	11.6 (±1.7) c	3.2 (±1.6) b			
<i>Correa reflexa</i> var. <i>cardinalis</i> (Labill.) Vent.	n.s.	0	0	-	-	-			
<i>Diplolaena dampieri</i> Desf.	***	8.1 (±0.8) b	44.2 (±3.8) c	1.2 (±0.7) a	12.4 (±2.6) b	6.2 (±1.8) b	■		27
	***	8.1 (±0.8) ab	44.2 (±3.8) d	2.4 (±1.3) a	27 (±6.4) c	12.6 (±4) b			
<i>Geleznowia verrucosa</i> Turcz.	***	0.4 (±0.4) a	43.5 (±2.6) c	0.9 (±0.9) a	12.8 (±3.3) b	10 (±1.8) b	■	ï	23
	***	0.4 (±0.4) a	43.5 (±2.6) b	2.2 (±2.2) a	35 (±10) b	26.3 (±5.4) b			
Santalaceae									
<i>Exocarpus sparteus</i> R.Br.		0.0 a	13.3 (±1.4) d	1.3 (±0.7) b	7.4 (±1.1) c	2.6 (±0.7) b			51
		0.0 a	13.3 (±1.4) c	4.9 (±2.8) b	29.7 (±4.9) d	9.9 (±2.9) bc			
<i>Leptomeria cunninghamii</i> Miq.	***	0.0 a	1.7 (±0.3) b	2 (±1.3) b	2.1 (±1) b	2.5 (±1) b			59
	*	0.0 a	1.7 (±0.3) a	26.7 (±17) b	26.5 (±13) b	33 (±13) b			
Solanaceae									
<i>Anthocercis littorea</i> Labill.	n.s.	0	0	0	0	0			35

Table 2.2 cont.

Taxon	P value	C	S	C/C	C/S	S/S	E.G	S.G	Days
Stackhousiaceae									
<i>Stackhousia pubescens</i> A. Rich	**	20.6 (±1.8) b	47.7 (±4) c	3.7 (±1.6) a	21.7 (±1.2) b	15.3 (±2.4) b	■		37
	**	20.6 (±1.8) b	47.7 (±4) c	8.7 (±3.9) a	52.2 (±2.1) c	53 (±4.5) c			
<i>Tripterococcus brunonis</i> Endl.	***	0.0 a	3.8 (±0.9) b	0.1 (±0.1) a	29.4 (±2.2) c	7.7 (±1.2) b	■	ï	25
	***	0.0 a	3.8 (±0.9) b	0.3 (±0.3) a	54.4 (±4) d	15 (±2.6) c			
Sterculiaceae									
<i>Lasiopetalum floribundum</i> Benth.	*	0.7 (±0.3) a	2.9 (±0.5) b	0.2 (±0.2) a	0.2 (±0.2) a	0.4 (±0.2) a		ï	41
	*	0.7 (±0.3) a	2.9 (±0.5) b	0.4 (±0.4) a	0.4 (±0.4) a	0.87 (±0.4) a			
<i>Thomasia glutinosa</i> Lindley	n.s.	0	0	0	0	0			
Styliaceae									
<i>Styloidium affine</i> Sonder	***	5.3 (±0.8) b	68.6 (±2.9) d	3.4 (±1.2) b	29.8 (±2.2) c	0.4 (±0.2) a	■	ï	29
	***	5.3 (±0.8) a	68.6 (±2.9) b	10 (±3.2) a	87.3 (±5.6) c	4.5 (±3.2) a			
<i>Styloidium amoenum</i> R. Br.	***	7.4 (±1.4) b	28.8 (±2.2) c	0.3 (±0.1) a	7.6 (±1.4) b	0.3 (±0.3) a			36
	***	7.4 (±1.4) b	28.8 (±2.2) c	0.9 (±0.3) a	20.5 (±3.4) c	0.9 (±0.9) a			
<i>Styloidium brunonianum</i> Benth.	***	13.8 (±1.3) b	44.6 (±5.8) c	0.1 (±0.1) a	11.6 (±3.2) b	0.1 (±0.1) a		ï	34
	***	13.8 (±1.3) b	44.6 (±5.8) c	0.3 (±0.3) a	29.4 (±7.9) bc	0.3 (±0.3) a			
<i>Styloidium bulbiferum</i> Benth.	***	3.9 (±0.9) a	15.9 (±0.7) b	7.1 (±1.7) a	26.8 (±2.2) c	4.6 (±0.7) a	■	ï	37
	***	3.9 (±0.9) a	15.9 (±0.7) b	15.7 (±4.3) b	59.9 (±5.6) c	11.7 (±1.7) b			
<i>Styloidium hispidum</i> Lindley	***	8.8 (±1) c	33 (±1.8) d	4.9 (±0.6) b	10.7 (±0.3) c	0.8 (±0.3) a	■	ï	29
	***	8.8 (±1) b	33 (±1.8) c	12.8 (±1.5) b	28 (±1) c	2.7 (±1.6) a			
<i>Styloidium junceum</i> R. Br.	***	11.2 (±1.5) b	82 (±3.7) d	9.3 (±1.4) b	27.6 (±2.3) c	2 (±0.7) a	■	ï	28
	***	11.2 (±1.5) a	82 (±3.7) c	27.4 (±3.7) b	78.5 (±6.1) c	19 (±4.2) ab			
<i>Styloidium schoenoides</i> DC.	***	3.8 (±0.7) a	55.2 (±2.6) d	2.9 (±0.6) a	32.9 (±2.7) c	8.7 (±1) b	■		41
	***	3.8 (±0.7) a	55.2 (±2.6) b	7.9 (±1.5) a	89.7 (±6.6) c	46.2 (±3.9) b			
Thymelaeaceae									
<i>Pimelea ciliata</i> B.L. Rye	***	0.0 a	10.3 (±0.9) b	0.3 (±0.2) a	12.3 (±1.6) b	9.6 (±1.7) b	■		33
	***	0.0 a	10.3 (±0.9) b	1.9 (±0.9) a	78.2 (±12) c	59.8 (±11) c			
<i>Pimelea imbricata</i> R. Br.	***	1.1 (±0.7) a	14 (±1.5) b	0.0 a	10.1 (±0.7) b	1.4 (±1.4) a	■		42
	***	1.1 (±0.7) a	14 (±1.5) b	0.0 a	69.8 (±5.4) c	9.3 (±9.3) ab			
<i>Pimelea leucantha</i> Diels	***	0.7 (±0.5) a	3.2 (±0.6) b	0.0 a	18.7 (±2.2) c	17.8 (±1.3) c	■		45
	***	0.7 (±0.5) a	3.2 (±0.6) a	0.0 a	33.7 (±4.7) c	31.5 (±2.7) c			
<i>Pimelea suaveolens</i> Meissner	**	0.0 a	23.3 (±3.3) b	0.0 a	6.6 (±6.6) a	6.6 (±6.6) a			45
	**	0.0 a	23.3 (±3.3) b	0.0 a	11.1 (±11) ab	11.1 (±11) ab			
Tremandraceae									
<i>Tetradlea hirsuta</i> Lindley	***	13.2 (±0.8) b	30 (±1.7) c	1.8 (±1) a	8.2 (±1.2) b	3.5 (±1) a	■	ï	43
	***	13.2 (±0.8) ab	30 (±1.7) bc	10.1 (±5.8) a	48.4 (±6.7) c	24.9 (±7.6) b			
Violaceae									
<i>Hybanthus calycinus</i> F. Muell	***	0.0 a	6.6 (±0.7) b	0.0 a	20.6 (±2.6) d	14.5 (±3.7) c		ï	38
	***	0.0 a	6.6 (±0.7) b	0.0 a	69 (±11) d	46 (±14) c			
<i>Hybanthus floribundus</i> (Lindley) F. Muell.	ns	0	0	-	-	-			

Table 2.3 The effects of smoke, method of smoke application and rapid ageing treatments on the seed germination of selected Australian native species. Data represent mean percent germination (\pm se) of the viable seeds within test replicates. Means subtended by different letters were determined to be significantly different (* = $P < 0.05$; ** = $P < 0.005$; *** = $P < 0.0005$; n.s. = not significant) by Tukeys multiple comparison test (HSD). C, control; S, seed smoked after sowing; ds, seed smoked prior to sowing; FR, fruit removed prior to sowing (C, control; S - smoked); NI, seeds nicked (C and S as before); L, seeds leached in running water (C and S as before); TR, seed coat removed (C and S as before); AC, acid treatment (C and S as before, time of immersion as indicated); WD, four wet/dry cycles prior to treatment where applicable (C and S as before). Where a hyphen (-) appears, the species was not subjected to the treatment.

Taxon	P Value	C	S	ds	FR C	FR S	NI C	NI S	L C	L S	TR C	TR S	AC C	AC S	WDC	WDS	
MONOCOTYLEDONAE																	
Anthericaceae																	
																	15 min.
	n.s.	0.0	0.5 (± 0.5)	-	-	-	1.5 (± 1.5)	4.5 (± 0.3)	1.5 (± 1.5)	1.5 (± 1.5)	-	-	1.5 (± 1.5)	1.5 (± 1.5)	0.0	0.0	
<i>Arthropodium strictum</i> Endl.	***	29 (± 2.5) a	59.3 (± 3.2) b	39.4 (± 4.5) a	-	-	-	-	-	-	-	-	-	-	-	-	
<i>Caesia calliantha</i> R.Br.	***	4 (± 0.9) a	10.9 (± 2) b	2.1 (± 0.6) a	-	-	-	-	-	-	-	-	-	-	-	-	
																	15 min.
<i>Johnsonia lupulina</i> R. Br.	n.s.	0	0	-	-	-	-	-	0	0	-	-	0	0	0	0	
<i>Sowerbaea multicaulis</i> E. Pritzel	n.s.	0.0	1.4 (± 0.8)	-	-	-	-	-	0.0	1.0 (± 0.6)	-	-	-	-	0.0	2.9 (± 1.2)	
Dasygogonaceae																	
<i>Acanthocarpus preissii</i> Lehm.	n.s.	0	0	-	-	-	0	0	-	-	-	-	-	-	0	0	
<i>Lomandra multiflora</i> R. Br.	**	1.6 (± 0.7) a	12.1 (± 2.2) b	6.2 (± 1.9) ab	-	-	-	-	-	-	-	-	-	-	-	-	
																	5 min.
<i>Lomandra preissii</i> (Endl.) Ewart	***	2.6 (± 0.5) ab	4.3 (± 0.7) ab	24 (± 7.4) c	0.8 (± 0.8) ab	6 (± 0.6) b	0.0 a	0.0 a	0.0 a	2.5 (± 1.1) ab	-	-	0.0 a	3 (± 1.2) ab	3.3 (± 1.7) ab	7.5 (± 1.7) b	
<i>Lomandra purpurea</i> (Endl.) Ewart	n.s.	14.4 (± 2.3)	21.8 (± 3.2)	17 (± 2)	-	-	-	-	-	-	-	-	-	-	-	-	
Haemodoraceae																	
<i>Macropidia fuliginosa</i> (Hook.) Druce	n.s.	0.0	0.0	-	-	-	-	-	0.0	1.5 (± 1.5)	-	-	-	-	0.0	0.0	
Phormiaceae																	
<i>Dianella revoluta</i> R. Br.	***	6.3 (± 0.9) a	34.7 (± 3.5) b	35.8 (± 4) b	-	-	-	-	-	-	-	-	-	-	-	-	
DICOTYLEDONAE																	
Apocynaceae																	
<i>Alyxia buxifolia</i> R. Br.	***	0.0 a	2.6 (± 1.2) a	-	0.0 a	8.1 (± 1.2) b	-	-	-	-	-	-	-	-	-	-	
Dilleniaceae																	
<i>Hibbertia commutata</i> Steudel	***	0.0 a	2.4 (± 0.9) ab	2.1 (± 1.2) ab	-	-	-	-	-	-	6.1 (± 0.9) b	21.7 (± 3.3) c	-	-	0.9 (± 0.9) a	2.6 (± 1.1) ab	
<i>Hibbertia lasiopus</i> Benth.	***	0.3 (± 0.3) a	11.8 (± 1.2) b	16.4 (± 2.1) c	-	-	-	-	-	-	8.6 (± 4.3) b	14.4 (± 2.4) bc	-	-	0.0 a	0.0 a	
<i>Hibbertia riparia</i> Hoogl.	***	1.1 (± 0.3) a	17.1 (± 0.9) c	9.1 (± 0.7) b	-	-	2.4 (± 0.9) a	12.3 (± 3.1) bc	-	-	-	-	-	-	-	-	
<i>Hibbertia sericea</i> Benth.	***	0.0 a	14 (± 4.2) c	6 (± 1.4) bc	-	-	1.6 (± 1.6) ab	12 (± 2) c	-	-	-	-	-	-	-	-	
<i>Hibbertia serrata</i> Hotchk.	**	0.0 a	1.9 (± 0.6) b	-	-	-	-	-	-	-	0.0 a	0.0 a	-	-	0.0 a	0.0 a	
Epacridaceae																	
<i>Acrotiche patula</i> R.Br.	***	0.0 a	0.7 (± 0.3) b	-	3.2 (± 0.4) c	13.8 (± 0.5) d	-	-	-	-	-	-	-	-	-	-	
																	30 min.
<i>Astroloma foliosum</i> Sonder	*	0.0 a	0.0 a	-	0.0 a	6.3 (± 3.9) b	-	-	-	-	-	-	0.0 a	2.7 (± 1.3) ab	0.0 a	0.0 a	
																	60 min.
<i>Astroloma pallidum</i> R.Br.	n.s.	0.0	0.0	-	0.0	1.3 (± 0.8)	0.0	0.0	-	-	-	-	0.0	0.0	0.0	0.0	
																	30 min.
<i>Leucopogon capitellatus</i> DC.	***	0.0 a	19.4 (± 2.5) b	-	-	-	-	-	-	-	-	-	0.0 a	16.8 (± 3.8) b	0.0 a	0.0 a	

Taxon	P Value	C	S	ds	FR C	FR S	NI C	NI S	L C	L S	TR C	TR S	AC C	AC S	WDC	WDS
60 min.																
<i>Leucopogon nutans</i> E. Pritzel	**	0.0 a	9.5 (±3) b	-	0.0 a	0.0 a	0.0 a	0.0 a	-	-	-	-	0.0 a	0.0 a	0.0 a	0.0 a
60 min.																
<i>Leucopogon propinquus</i> R. Br.	n.s.	0	0	-	0	0	0	0	-	-	-	-	0	0	0	0
<i>Leucopogon verticillatus</i> R. Br.	n.s.	0	0	0	-	-	-	-	-	-	-	-	-	-	-	-
<i>Styphelia tenuiflora</i> Lindley	n.s.	0	0	-	0	0	0	0	-	-	-	-	0	0	0	0
Goodeniaceae																
<i>Brunonia australis</i> Smith	***	4.1 (±1.2) a	20.2 (±1.8) b	25.5 (±1) b	-	-	-	-	-	-	-	-	-	-	-	-
<i>Scaevola calliptera</i> Benth.	***	0.0 a	13.8 (±2.4) b	-	-	-	0.0 a	13.4 (±3.3) b	-	-	-	-	-	-	-	-
4 min.																
<i>Scaevola crassifolia</i> Labill.	***	33.8 (±2.4) b	47.3 (±2.6) bc	72.6 (±6.8) cd	-	-	-	-	-	-	-	-	54.7 (±4.5) bcd	83.4 (±3.5) d	2.1 (±1) a	51.8 (±14) bc
4 min.																
<i>Scaevola fasciculata</i> Benth.	***	0.0 a	3.3 (±0.3) b	-	-	-	-	-	-	-	-	-	0.0 a	20.6 (1.7) c	0.0 a	4.6 (1.2) b
Myrtaceae																
<i>Calytrix fraserii</i> Cunn.	***	4.4 (±0.9) b	12.7 (±1.6) c	-	-	-	-	-	-	-	-	-	-	-	0.0 a	0.0 a
<i>Calytrix tetragona</i> Labill.	***	12.8 (±1) b	25.7 (±2.5) c	19.1 (±3.1) bc	-	-	-	-	-	-	-	-	-	-	0.0 a	0.0 a
Papilionaceae																
<i>Hovea chorizemifolia</i> (Sweet) DC.	***	51 (±3.7) b	66.6 (±2.2) c	24 (±2.4) a	-	-	-	-	-	-	-	-	-	-	-	-
Pittosporaceae																
Seeds extracted from Pods																
<i>Billardiera varifolia</i> DC.	***	0.0 a	0.9 (±0.5) a	-	8.4 (±1.5) b	50.5 (±1.8) c	-	-	-	-	-	-	-	-	-	-
<i>Bursaria spinosa</i> Cav.	***	31.8 (±1.5) c	16.2 (±1.1) b	6.6 (±0.9) a	-	-	-	-	-	-	-	-	-	-	-	-
Proteaceae																
<i>Conospermum incurvum</i> Lindley	***	0.0 a	5.3 (±0.8) b	-	-	-	-	-	-	-	-	-	-	-	0.0 a	0.6 (±0.3) a
<i>Conospermum triplinervium</i> R. Br.	***	0.0 a	2.9 (±0.6) b	-	-	-	-	-	-	-	-	-	-	-	0.0 a	2 (±0.7) b
<i>Isopogon ceratophyllous</i> R. Br.	***	0.0 a	22.2 (±5.1) b	35.1 (±4) c	-	-	-	-	-	-	-	-	-	-	-	-
30 min.																
<i>Persoonia elliptica</i> R. Br.	n.s.	0	0	-	-	-	0	0	-	-	-	-	0	0	0	0
60 min.																
<i>Persoonia longifolia</i> R.Br. (laboratory stored, < 1 year since collection)	n.s.	0	0	-	0	0	0	0	-	-	-	-	0	0	0	0
Rhamnaceae																
<i>Pomaderris obcordata</i> Fenzl	n.s.	0.4 (±0.1)	0.5 (±0.1)	0.3 (±0.1)	-	-	-	-	-	-	-	-	-	-	-	-
<i>Spyridium parvifolium</i> F. Muell. L.	**	0.2 (±0.1) a	1.2 (±0.2) b	1.3 (±0.1) b	-	-	-	-	-	-	-	-	-	-	-	-
Rutaceae																
30 min																
<i>Boronia megastigma</i> Pl. Preiss	n.s.	0	0	0	-	-	0	0	0	0	-	-	0	0	0	0
30 min.																
<i>Boronia viminea</i> Lindley	**	1.1 (±0.2) a	4.4 (±1.2) b	-	-	-	-	-	0.3 (±0.3) a	0.6 (±0.4) a	-	-	0.0 a	0.2 (±0.2) a	0.0 a	0.4 (±0.2) a
<i>Geleznowia verrucosa</i> Turcz.	***	0.4 (±0.4) a	43.5 (±2.6) d	30.7 (±3.8) d	-	-	-	-	0.9 (±0.9) ab	4.3 (±1.3) b	-	-	-	-	3.6 (±1) b	13.7 (±1.5) c
Santalaceae																
<i>Exocarpus sparteus</i> R.Br.	***	0.0 a	13.3 (±1.4) b	-	-	-	-	-	-	-	-	-	-	-	0.0 a	0.0 a
Stackhousiaceae																
<i>Stackhousia pubescens</i> A. Rich	**	20.6 (±1.8) b	47.7 (±4) c	-	-	-	-	-	-	-	-	-	-	-	0.0 a	0.0 a

CHAPTER 3. SITES OF ACTION OF SMOKE IN BREAKING SEED DORMANCY

3.1 INTRODUCTION

Plant-derived smoke enhances germination of many Australian plant species that would otherwise not germinate under standard nursery or field conditions (Roche, 1993; Dixon *et al.*, 1995). Ascertaining the mechanism by which smoke breaks dormancy will benefit the horticultural industry as high germination rates of Australian species may be achieved by determining such things as time of application and quantity of smoke per application. The importance of finding the mechanism by which smoke has the ability to relieve dormancy has a considerable significance due to the knowledge that the dormancy of species from non fire-prone environments can be broken by exposure to smoke (Pierce *et al.*, 1995; Drewes *et al.*, 1995). In a recent study, it was found that smoke could replace the light requirement in breaking dormancy of lettuce, *Lactuca sativa* L. (Drewes *et al.*, 1995). Therefore elucidation of the dormancy breaking mechanism of smoke will have wider horticultural and possibly agricultural implications.

Determining target tissues in seed which are the key sites of action is imperative to an understanding of the mechanisms by which smoke breaks dormancy of seeds. As smoke does not break dormancy by physically degrading the seed coat or play a nutritive role (Baldwin *et al.*, 1994) it is more likely that smoke acts as a chemical functionary. These findings indicate a possible interaction of smoke chemicals with inhibitors either from the seed coat, endosperm, embryo or combinations of these seed tissues.

The presence of seed coat inhibitors can be determined the by the removal of the seedcoat to overcome dormancy or by reimposing dormancy by replacing the seedcoat with the excised embryo (Jackson, 1968). Inhibitors in seeds of Western Australian species such as *Eremophila* (Richmond and Ghisalberti, 1994), *Lomandra sonderi* (Plummer *et al.*, 1995) have been found in pericarps, fruit tissues of seeds and in seed coats of *Actinotus helianthii* (Lee and Goodwin, 1993) which when removed, relieved seed dormancy. It is therefore hypothesised that smoke may act in a similar fashion on seed coat inhibitors to relieve

dormancy on the two species selected for study, *Stylidium affine*, an indicator species, and *Actinotus leucocephalus*. *A. leucocephalus* is a species suitable for use as an annual bedding plant, however, it germinates erratically and at low levels under standard nursery conditions.

Growth hormones such as gibberellic acid (GA₃) and cytokinins (zeatin) are well known for their ability to overcome inhibitors and promote germination (Hillhorst and Karssen, 1992; Bell *et al.*, 1993; Bewley and Black, 1995) by GA controlled endosperm hydrolysis which reduces mechanical restraint imposed on the embryo and mobilisation of food reserves (Hillhorst and Karssen, 1992) and enhanced embryo growth by cytokinin.

This study aimed to determine: (a) the location of germination inhibitors in the seed of the 2 species studied; and (b) to compare smoke with the dormancy breaking hormones, GA₃ and zeatin, in its effectiveness in enhancing germination.

3.2 MATERIALS AND METHODS

3.2.1 Seed tissue responses of *Stylidium affine* to smoke (I)

Seeds of all species were purchased from Nindethana Seed Company except for *A. leucocephalus* which was bush picked from wild sites east of Forrestania (350 km SE of Perth, Western Australia) and stored for 6 months prior to testing.

Seeds of all study species were vacuum sterilised in sodium hypochlorite solution (1 %) (5 min on, 5 min off and 5 min on) and then transferred to sterile conditions. Seeds were then rinsed in sterile distilled water (1.5 L) or until all traces of bleach was removed (Meney and Dixon, 1988). The seeds were then left to imbibe at room temperature (23° C) for 24 hours in sterile polycarbonate tubes. Seeds were imbibed to facilitate removal of seedcoats. Under sterile conditions, five replicates of 10 seeds treated as in Table 3.2. were placed onto two layers of 7cm sterile filter paper (No. 1 Whatman) with 1.5 ml of one of four nutrient and smoke solutions.

The treatment solutions were made up using half strength MS solution (components listed in table 3.1) made up to 1 L. PH of the media was adjusted to 6 and sterilised. Gibberellic acid

(GA₃, 1ml) and zeatin (1 ml) were filter sterilised and added to 1/2 strength MS solutions when treatment was required in sterile conditions. Media containing smoked water was prepared by adding 100 ml of full strength smoked water to 900 ml of 1/2 strength MS solution. All solutions were adjusted to a pH of 6.

Table 3.1 Components of 1/2 MS Solution

Component	Amount g L ⁻¹
Sigma® plant culture Murashige and Skoog basal salt mix (2L)	43.3
Ferric sodium salt=FeEN ^a (EDTA)	0.367
MuO Inositol	1.84
Thiamine HCl	0.034
Pyridoxine HCl	0.021
Niacin	0.013

Table 3.2 Seed and media treatments

Seed treatment	Nutrient and treatment solution(1.5 ml)
Control (whole seed)	1/2 MS
Seed with seed coat removed	1/2 MS + GA ₃ (3 ml L ⁻¹) + zeatin (1 ml L ⁻¹)
Excised embryo	1/2 MS + 10% SW
	1/2 MS + 10% SW + GA ₃ (3 ml L ⁻¹) + zeatin (1 ml L ⁻¹)

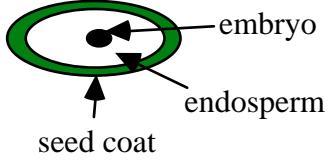

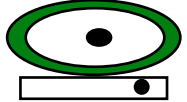

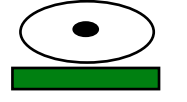

Seeds in petri dishes were sealed with plastic wrap to prevent contamination and moisture loss. Petri dishes were placed in a complete random block design, wrapped in aluminium foil and stored in a darkened incubator at 19°C for 7 weeks. Germinated seeds were counted once a week from the third to seventh week.

3.2.2 Seed tissue response to smoke of *Stylidium affine* (II)

This experiment is an extension of the first experiment and aims to collect further evidence on the responsiveness of the seed coat to smoke. This was achieved by firstly reimposing the seed coat (which was found to be inhibitory in the first Experiment) onto the endosperm and embryo and secondly, a seed coat onto intact whole seed. *S. affine* seeds were sterilised using the vacuum method (section 3.2.1) and soaked for 1 h in sterile distilled water. Seeds were treated 6 different ways including the control (Table 3.3). Five replicates of 10 seeds were prepared for each treatment as listed in Table 3.3 and placed on two layers of 7 cm sterile

filter paper (No. 1, Whatman) in petri dishes with 1.5 ml of two nutrient and smoke solutions (Table 3.3) as prepared in Section 3.2.1.

Table 3.3 Seed and media treatments

Seed treatment	Nutrient and treatment solution(1.5 ml)	
	Control (whole seed)	
	Seed + seedcoat ^a	1/2 MS ^d
	Seed + 'intact endosperm+embryo' ^b	
	Nicked seed	1/2 MS + 10% SW
	Seed without seedcoat' + seedcoat ^c	
	Seed with seed coat removed	

^a intact seed was placed with one excised seed coat

^b intact seed was placed with one seed with its seedcoat removed

^c seed with its seedcoat removed was placed on top of one excised seedcoat

^d Murashige and Skoog (1962) solution

Petri dishes were sealed with plastic wrap to maintain moisture and prevent contamination. Petri dishes were placed in a complete random block design, wrapped in aluminium foil and stored in a darkened incubator at 19°C for 7 weeks. Germination and embryo growth was recorded from week three to seven.

3.2.3 Seed tissue response to smoke of *Actinotus leucocephalus*

A similar trial was conducted on *A. leucocephalus* as described for *S. affine* (section 3.2.1). Seeds were sterilised as outlined in section 3.2.1. Five replicates of 10 seeds, either whole or

with their seedcoat removed (pericarp and testa) were placed on two layers of 7cm filter paper (No 1. Whatman) in Petri dishes with 1.5 ml 1/2 MS or 1/2 MS + 10% SW (Table 3.2). Seeds were wrapped in aluminium foil and stored in a darkened incubator at 19°C. Germination was recorded from week three to seven.

3.2.4 Statistical analysis

Germination results were transformed to percentages and adjusted for viability by multiplying raw data by the viability of each species. Least significant differences (LSD) between the control and smoked water treatments at 5% confidence level was determined by Analysis of Variance (ANOVA). Data that were not normally distributed were transformed by Angular Arcsine Transformation and their ANOVA's performed.

3.3 RESULTS

3.3.1 Seed tissue response to smoke of *Stylidium affine* (I)

A quarter of seeds without seed coats germinated in water compared to zero germination recorded by whole seeds. When treated with smoked water, about 50 % of whole seed and seeds without seed coats germinated. When treated with GA₃ and zeatin, whole seeds did not germinate whereas approximately 75 % of seeds whose seed coats had been removed germinated. Similarly, 75 % of whole seeds and seeds without seed coats germinated when exposed to a solution of smoked water, GA₃ and zeatin (Fig. 3.1). After 7 weeks, embryos in the four treatments had not.

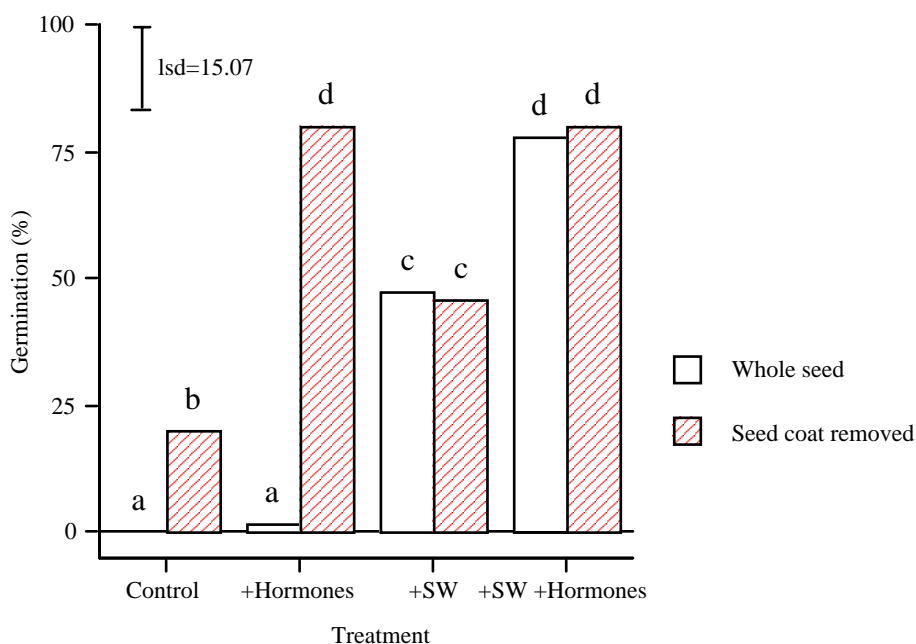


Fig. 3.1—Germination (%) of whole *S. affine* and seeds with seed coat removed after 7 weeks in 1/2 MS on filter paper in Petri dishes with no treatments (Control) or GA₃ (3 ml L⁻¹) and Zeatin (1 ml L⁻¹) (+Hormones) or 10% SW or GA₃ (3 ml L⁻¹) and Zeatin (1 ml L⁻¹) & 10 % SW (+SW +Hormones) (1 ml L⁻¹). Values followed by the same letter are not significantly different ($p < 0.05$).

3.3.2 Seed tissue response to smoke of *Stylidium affine* (II)

Very low germination was recorded for 'seeds without seed coats' in contact with 'dissected seed coat' in water. When incubated on smoked water, two thirds of this seed treatment germinated. The control, 'seed with seed coat' and 'seed with endosperm' did not germinate in water. In comparison, two thirds of seeds in these treatments and exposed to smoked water germinated.

The impact of seed coat removal on smoke mediated germination showed that the greater the proportion of seed coat removed, the higher the germination percentage. In water, seeds with whole seed coat removed had a germination percentage of 42 % and nicked seeds 24 % germination in water and on smoked water, 56 and 58 % respectively (Fig. 3.2).

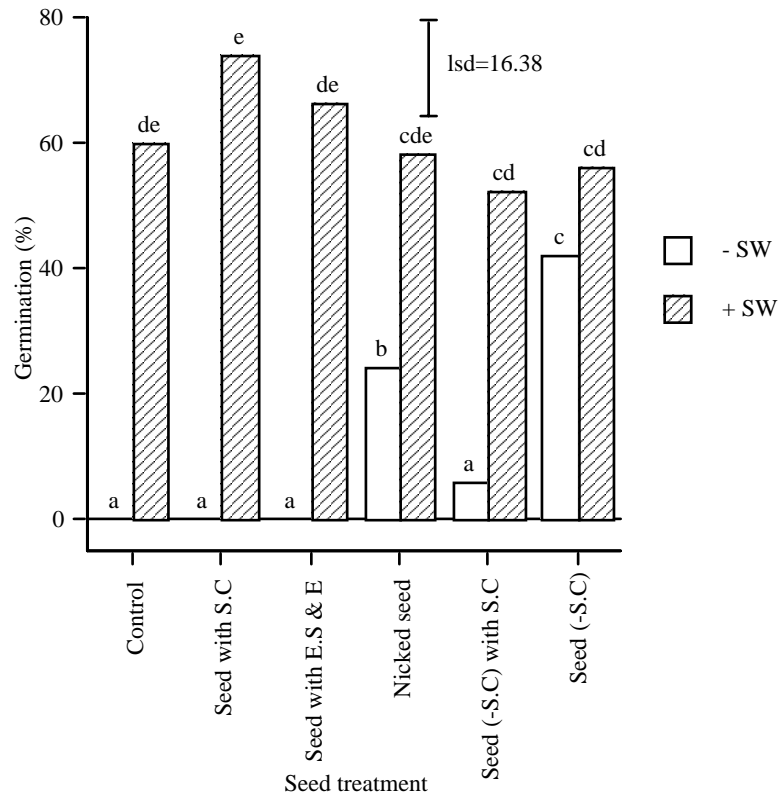


Fig. 3.2—Germination (%) of *S. affine* after 7 weeks on 1/2 MS on filter paper in Petri dishes with no treatments (Control) or whole seed with seedcoat (Seed on S.C.) or whole seed with intact endosperm (E.S.) and embryo (E) (Seed with E.S. & E) or seed with seed coat removed with dissected seed coat (Seed (-S.C.) on S.C. or nicked seed with smoked water (+SW), seed with seed coat removed (Seed (-S.C.)) with or without (-SW). Values followed by the same letter are not significantly different ($p < 0.05$).

3.3.3 Seed tissue response of *Actinotus leucocephalus* to addition of smoke

Whole seed of *A. leucocephalus* did not germinate in water and removal of the seed coat did not substantially increase germination. Germination of whole seeds on SW was slightly higher but was significantly greater when seed coats were removed (Fig. 3.3).

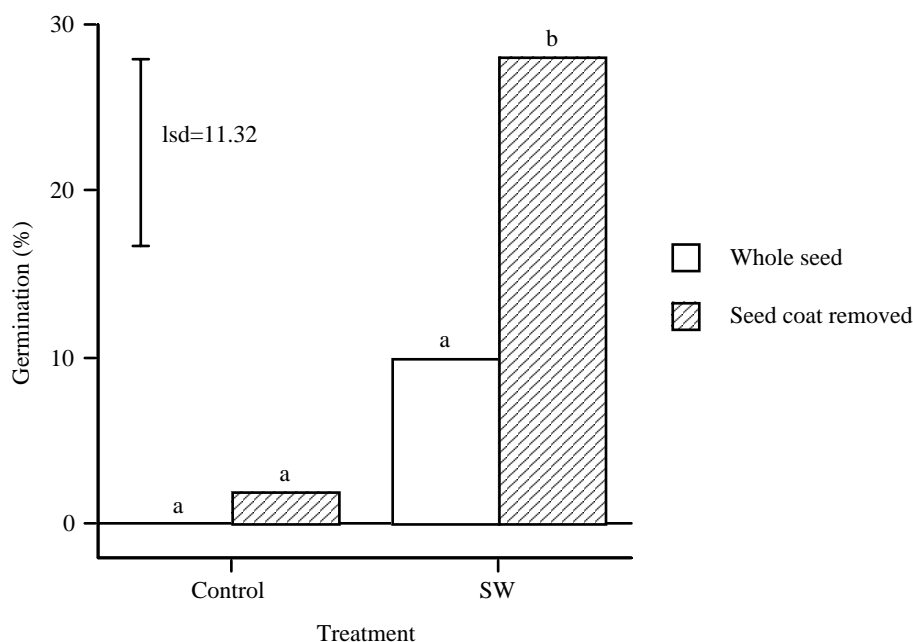


Fig. 3.3—Germination (%) after 7 weeks of *A. leucocephalus* whole seed or with seed with coat removed in presence or absence (control) of smoked water (SW). With all treatments incubated on 1/2 MS. Values followed by the same letter are not significantly different ($p < 0.05$).

3.4 DISCUSSION

Germination of *Stylidium affine* was partly inhibited by its seed coat (Fig. 3.1) as germination was significantly enhanced when the seed coat was removed. When SW was applied, germination of whole seed was greatly enhanced which suggested that smoke cues overcame dormancy by acting on the seed coat in *S. affine*. Further evidence to support the hypothesis was that inhibition of germination when the seed coat was reimposed in contact with 'endosperm and embryo' incubated in water (Fig. 3.2). However, when SW was applied, dormancy was relieved, apparently overcoming inhibitors in the seed coat. From these results, it may be deduced that smoke does not break dormancy by overcoming physical barriers in seed coats but rather SW acts by reducing inhibitory factors in the seed coat. Further, these results also show that the seed coat of *Stylidium affine* does not impose seed dormancy by preventing the outward movement of inhibitors as was found in *Emmenanthe penduliflora* by

Egerton-Warburton (1997, unpubl.). This finding also supports results of Baldwin *et al.*, (1994). The inhibition influence of the seed coat was also noted by the gradual removal of the seed coat. With intact seed coats, there was no germination and with a nicked seed, germination increased to 20 % with further improvement in germination in 40 % when the whole seed coat was removed (Fig. 3.2).

Smoke therefore appears to overcome dormancy by acting on the seed coat which was found to be chemically inhibitory. Smoke may overcome inhibitors in the seed coat in a number of ways. Firstly, Baldwin *et al.* (1994) suggested that smoke induced germination was consistent with signal-mediated mechanisms. This may mean that receptors located in the seed coat receive smoke cues and signal processes to occur, culminating in germination. Smoke extracts may also neutralise seed coat inhibitors by triggering reactions leading to metabolism of inhibitors. This will favour promotor to inhibitor ratio and induce germination. This theory also explains why GA₃ and zeatin could not overcome dormancy of whole seeds and only could when the seed coat was removed.

The whole seed of *S. affine* seed did not germinate in GA₃ and zeatin unless the seed coat was removed (Fig. 3.1). Removal of the seed coat therefore appears to have removed inhibitors so that GA₃ and zeatin could act on the endosperm and embryo to enhance germination. As GA₃ and zeatin treatment alone was not able to overcome dormancy imposed by the seed coat, it also provides support for the hypothesis that smoke acts on the seed coat or residing chemicals to relieve dormancy. It also suggests that GA₃ and zeatin enhances germination via the endosperm and embryo which has been widely acknowledged to be so (Groot and Karsen, 1987; Hillhorst and Karson, 1992).

An additive effect of smoke and hormones on germination was observed in *S. affine* (Fig. 3.1). This clearly indicates a synergistic effect of hormones on smoke. This observation may provide the first clear quantification of the hormone like action of smoke as smoked water gave a similar result to hormones under certain treatment conditions in an Australian species. The synergistic effect of GA₃ and zeatin on smoke may therefore explain why residual viable seed banks remain after normal germination and smoke induced germination. This study has horticultural and ecological implications as it indicates a possible method to germinate this residual seed bank and possible agent(s) involved in residuality in seed banks.

As illustrated, evidence was found to support the hypothesis that smoke acts on the seed coat to relieve seed dormancy. However, it was also shown that smoke may also act on the endosperm and/or embryo to break seed dormancy. This was supported by the higher germination of 'seeds without seed coat' in SW over the same seed treatment in water. If it was assumed that the seed coat controls dormancy, germination of 'seeds without seed coat' in SW should be the same as that found in water, which it was not and therefore does not support the hypothesis that smoke breaks dormancy by solely acting on the seed coat (Fig. 3.1).

The experiments suggest that the mode by which GA₃ and zeatin break dormancy was probably not the same or not as effective on the same dormancy sites as SW. This was demonstrated by the ineffectiveness of GA₃ and zeatin to break the dormancy of whole seed compared to smoked water. Instead the GA₃ and zeatin treatment was only effective on seeds without their seed coat. This observation suggested that GA₃ and zeatin could not overcome the inhibitors of the seed coat while SW capable of doing so.

In contrast for *A. leucocephalus*, smoke was found to act on the endosperm and/or the embryo but not on the seed coat as was the case with *S. affine*. The removal of the seed coat did not enhance germination, indicating that the inhibitory mechanism was located either in the endosperm and/or the embryo and that smoke overcame dormancy in those tissues (Fig. 3.3). This was further demonstrated by the high germination of seeds without seed coats in smoked water. It therefore appears that seed coat inhibition was not operating on *A. leucocephalus*. However, seed coat imposed dormancy was demonstrated in the East Australian species, *Actinotus helianthi* (Lee and Goodwin, 1993). As the two species are endemic to different parts of Australia, thus belong to different provenances, this may explain the apparent differences in their dormancy mechanisms as well as other factors that can occur from the growth of parent plant, harvesting, storage and distribution.

Results presented in these experiments indicate that smoke can play an active role in overcoming dormancy in seed of Western Australian species. It appears that germination inhibitors are located in different tissues of the seed of different species and smoke acts on these tissues to overcome dormancy. In addition, for some species, the action of smoke can be significantly enhanced in concert with certain promotive plant hormones. The mode of action

of the synergism and the benefits of promotive hormone supplements in stimulating germination of residual seed banks have important ecological and horticultural implications for future applications of smoke technology.

CHAPTER 4. OVERCOMING GERMINATION DIFFICULTIES IN *BORONIA MEGASTIGMA* NEES EX BARTLING.

4.1 INTRODUCTION

Boronia megastigma Nees. ex Bartling is a shrub native to the jarrah forest in the southwest of Western Australia. This species has considerable horticultural potential as it contains valuable essential oils (Plummer 1996). The plant is grown readily from cuttings, however, cultivation from seed is fraught with difficulty. The development of superior genotypes by breeding has been limited by difficulties in seed germination (Plummer 1996; Plummer et al 1997). Few seeds germinate due to poor viability and seed dormancy (Johnson and Burchett 1996).

In the wild the species is killed by fire but seedlings readily recruit in the post-fire environment. It was suspected that this may be a germination response to smoke stimulation. Success with fire-stimulation of seed germination has been reported by commercial *Boronia* growers (Maguire 1993) but recent attempts with direct smoke-stimulation of *Boronia megastigma* seed have been unsuccessful (Dixon *et al.* 1995). Although fire has been observed to stimulate an impressive amount of germination in the field, Plummer and Haddon (pers. observation) estimated that the level of emergence only represents 1-2% germination based on estimates of annual seed production.

Roche *et al.* (1997) found that ageing the seed in the soil for 1-2 years followed by smoke treatment was able to stimulate some germination (up to $12.2 \pm 1.8\%$). Storage of seed in the soil for one year coincided with a decline in seed viability from 93% to 45% which compromises recruitment of the species. A range of rapid ageing methods (nicking, leaching, acid scarification and wet-dry cycles), applied alone and together with smoke treatment, failed to simulate the germination achieved in soil stored seed. Soil storage in combination with smoke treatment is a key to achieving germination in this species.

Initial work at Kings Park and Botanic Gardens on this species using two year old seed failed to produce any germinants, even though almost 100% of seed appeared to contain a full and

healthy endosperm when sectioned. Embryos extracted from the seed and grown on half MS media with GA₃ and zeatin or smoke water failed to show any growth response. This would suggest that the embryos are deeply dormant. This result was also confirmed by Plummer *et al.* (unpubl.) who found that seed stored for 12 months at 4°C could not be germinated even when scarified or treated with GA₃, fire or smoke.

Recent work by Plummer *et al.* (unpubl.) found that *in vivo* germination only occurred for seed removed from fruit harvested 82 days post anthesis (DPA) which was soaked (6 h) in gibberellic acid (GA₃, 250 mg L⁻¹) and then dried (20°C, 48 h) to release seed. Very few (1%) intact seed germinated *in vitro* and these were removed from fruit harvested <25 DPA. Most excised embryos (84±16%) or decoated seeds (60±1%) harvested 82 DPA grew *in vitro*. By 87 DPA many embryos had aborted and viable embryos (30±10%) were dormant. It was concluded that separate dormancy mechanisms exist in *Boronia* seed coats, embryos and possibly endosperm tissues.

The following study aimed to investigate ways of overcoming germination difficulties in *Boronia megastigma*. This involved: a study of natural seed banks; an examination of the change in germinability of *Boronia* seed leading up to and following dehiscence (following the findings of Plummer *et al.* unpubl.); and investigations of cryopreservation of the species to allow it to be stored prior to the onset of inhibitors. It is hoped that an improvement in germination success can be achieved to allow a reasonable proportion of the progeny from an artificial cross to be germinated in a breeding programme.

4.2 METHODS

4.2.1 Study site

A natural *Boronia megastigma* stand was located in Mersea State Forest, 12km south of Bridgetown (along Conto Road). The site had been burnt 4 years prior to the study and adult *Boronia* plants were relatively numerous along a creekline.

4.2.2 *In situ* seedbank study

Eight Topsoil cores 20x20x5cm were collected in the vicinity of adult plants in early May. The soil was sieved and any *B. megastigma* seed was removed, counted and germinated (with seed coat removed) in tissue culture tubes of 1/2 MS with GA₃ and zeatin using the techniques described below.

In early May, prior to the onset of winter rainfall, six randomly placed 2x2m plots were pegged out in the Boronia site close to adult plants. Three of these plots had aerosol smoke applied directly to the topsoil *in situ* using the procedure described in section 1.3.2. Three were left untreated as controls. In late spring these plots were scored for the number of Boronia seedlings present.

4.2.3 Germination studies

To test the change in germinability of Boronia seed following anthesis, seed was collected from several plants at the study site on four occasions prior to dehiscence (23/10/97, 30/10/97, 10/11/97 and 28/11/97). Prior to dehiscence stocking material was placed over fruits to collect seed as it was shed. Dehiscence occurred some time between 28/11/97 and 13/1/98 (when the dehisced seed was collected).

A similar experiment was carried out using potted plants in the glasshouse. Ten plants (var. Brown Boronia), purchased from a commercial nursery, were hand pollinated. The developing fruits were then harvested on four occasions prior to dehiscence (23/10/97, 30/10/97, 12/11/97 and 15/12/97). Unfortunately, flowering success was variable in these plants and there was not sufficient seed to collect after dehiscence.

The seed collected from Boronia plants was physically removed from its fruit and surface sterilized (following section 3.2.1). The seed was rinsed in sterile water. Seed was placed onto a filter paper bridge in tissue culture tubes containing 10ml of 1/2MS solution (see section 3.2.1) with 3ml/L of GA₃, 1ml/L of Zn and 0.75g/L of Benlate solution®. Generally 5 seeds were placed in each tube and the number of replicates for each treatment varied depending on the availability of seed. Initially two treatments were applied to the seed: whole seed; or seed

which had their seed coat nicked. Later, when it was determined that intact seed did not germinate, all seed was nicked.

To determine changes in germinability after collection the dehisced batch of wildpicked seed was germinated *in vitro* after two time periods of storage (16/3/98 and 30/3/98). This seed was stored in an airtight container refrigerated at 4°C.

In order to determine if *B. megastigma* seed was capable of imbibing water three replicates of 50 seed (dehisced batch) were weighed and then placed in distilled water for a week. To determine the rate of imbibition the seed samples were removed, patted dry with paper towels and weighed at regular intervals over a week before being returned to the water.

4.2.4 Cryostorage

To overcome the rapid viability decline in *Boronia* seed cryopreservation was investigated as a means of arresting any changes in seed development. In an initial trial wild-picked seed (collected 28/11/97) was placed into a polyethylene cryovial (Nunc®) and plunged directly into liquid nitrogen. After 3 days storage the vial was removed from liquid nitrogen, thawed at room temperature and then germinated using the method described above (7 replicates of 5 seed, all seed was nicked).

In a later trial dehisced seed was used and the following pretreatments were applied (with 5-7 replicates of 5 seed used in each treatment) prior to cryostorage:

(i) 30 untreated seed was germinated directly (control);

(ii) 100 seed was dried in LiCl₂ solution with a relative humidity of 13% for 4 days. 40 of these seed were germinated directly. Another sample of 50 seed were placed in liquid nitrogen, removed after 48 hours, and then germinated. The remaining sample of 10 seed were tested for moisture content using the Karl-Fischer Titration method; and

(iii) 30 seed was placed in a cryoprotective solution comprising of 15% (w/v) ethylene glycol, 15% (w/v) dimethyl sulfoxide and 30% (w/v) glycerol in 1/2MS nutrient media supplemented with 0.4M sucrose for 30 minutes before being plunged into liquid nitrogen.

All treatments were surface sterilized, seedcoats nicked and germinated in tissue culture using the methods described above. The 1/2 MS media used in this trial did not contain the hormones GA₃ or zeatin.

4.3 RESULTS

4.3.1 *In situ* seedbank study

A density of 121.9 ± 50.8 seeds/m² was determined from *B. megastigma* seed collected from the soil cores taken from the study site. Of the 39 seeds collected 40.9% germinated *in vitro*. A density of 50.0 ± 20.8 germinable seeds/m² can be estimated based on the two previous figures. The soilstored seed removed from soil cores were highly scarified in appearance, having lost the shiny black lustre, evident in fresh seed.

Aerosol smoked plots yielded 4 ± 1.9 germinants/m² whilst there were no germinants in the controls. This density of germination is significantly lower than the estimate of germinable seeds/area determined from the soil cores.

4.3.2 Germination studies

Seed collected from glasshouse plants produced no germinants in the first two batches of seed picked (about 2 months prior to dehiscence) and no germination occurred from whole seed in any batch from glasshouse plants. Germination did occur from nicked treatments in the third batch ($77.8 \pm 10.2\%$, 6 replicates of 2-3 seed), fourth batch ($96.7 \pm 3.3\%$, 6 replicates of 2-5 seed) and the final batch ($82.2 \pm 9.7\%$, 3 replicates of 3-5 seed).

There was no germination recorded for any of the first two batches of seed collected from the study site and no whole seed germinated from any of the batches collected. Germination did occur in nicked treatments of the third batch ($6.7 \pm 6.7\%$, 3 replicates of 5-7 seed) and the fourth batch ($27.8 \pm 7.1\%$, 14 replicates of 4-5 seed). After being stored in the fridge for 4 weeks the germinability of nicked seed had dropped to $3.6 \pm 3.6\%$ (7 replicates of 3-5 seed) and a cut test revealed that only $0.7 \pm 0.7\%$ of seed contained a full endosperm of healthy appearance, the majority of seed appeared to contain a dehydrated endosperm of ßflouryí

appearance or one that had shrivelled in size. Of the post-dehisced seed there was $83.8\pm 6.4\%$ germination (17 replicates of 5 seed, all nicked). This level of germination reflected the results of a cut test which showed $74.7\pm 3.3\%$ of seeds contained a full endosperm of healthy appearance.

The germinability of predehisced seed declined from $27.8\pm 7.1\%$ at the time of collection to $3.6\pm 3.6\%$ after four weeks storage at 4°C . Seed collected after dehiscence also declined from $83.8\pm 6.4\%$ germination at the time of collection to $60.0\pm 12.6\%$ after 8 weeks and to $28.0\pm 4.4\%$ after 10 weeks.

Seed soaked in water increased in weight by $79.9\pm 15.9\%$ over a period of one week.

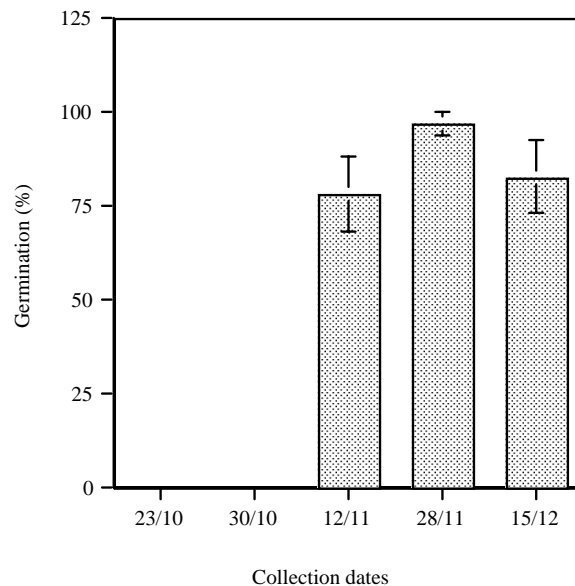


Fig. 4.1—*In vitro* germination of *Boronia megastigma* seeds collected at four different intervals (numbers represent day/month) prior to dehiscence from plants grown and pollinated in a glasshouse. The data represents nicked treatments only as whole seed did not germinate.

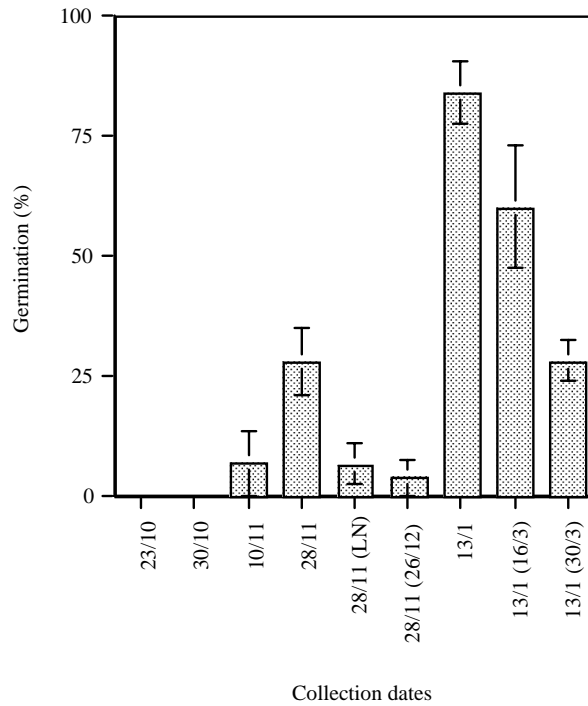


Fig. 4.2—*In vitro* germination of *Boronia megastigma* seeds collected at four different intervals (numbers represent day/month) prior to dehiscence and one interval after dehiscence (13/1/98) from a wild population. A sample of the fourth batch was stored in liquid nitrogen (LN) prior to storage and another was germinated after a period of storage at 4°C (germinated 26/12). The fifth batch was germinated immediately after collection and at two later dates after storage (16/3 and 30/3). The data represents nicked treatments only as whole seed did not germinate in any treatment.

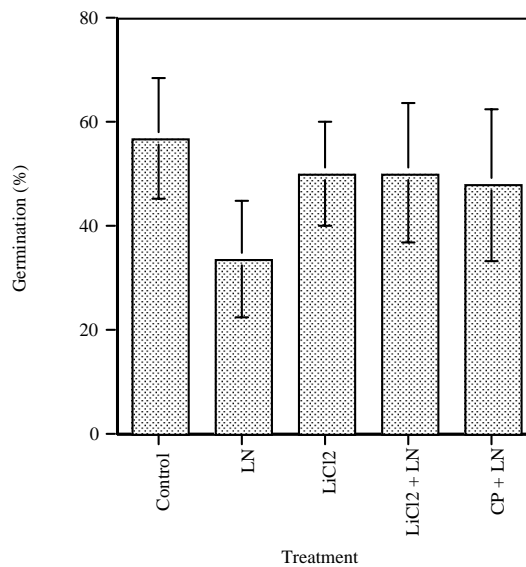


Fig. 4.3—*In vitro* germination of *Boronia megastigma* seed after five treatments relating to cryostorage: control, germinated directly; LN, placed in liquid nitrogen storage prior to germination; LiCl₂, placed in lithium chloride for 4 days prior to germination; LiCl₂ + LN, placed in lithium chloride for 4 days followed by storage in liquid nitrogen prior to germination; and CP + LN, placed in a cryoprotectant solution for 30 minutes prior to germination.

4.3.3 Cryostorage

The initial seed trial placed in cryostorage (wildpicked batch 4) produced $6.4 \pm 4.18\%$ (7 replicates of 4-5 seed) germination compared with $27.8 \pm 7.1\%$ germination in unfrozen seed. The moisture content of this batch of fresh seed was 17.5%.

In the later trials it was found that there was no significant difference in the level of germination between the control (unfrozen seed) ($56.57 \pm 11.48\%$) and any of the cryopreservation treatments (Fig. 4.3). Seed that was placed directly in liquid nitrogen without a pretreatment to reduce its moisture content was not significantly lower ($33.33 \pm 11.16\%$) than pretreated seed or untreated seed, unlike the result of the previous trial. Seed pretreated with LiCl₂ had its moisture content reduced to 7.093%. and there was no significant difference between the germination response of frozen ($50.0 \pm 13.4\%$) and unfrozen ($50.0 \pm 10.0\%$) seed. Germination of seed treated with the cryoprotective solution ($47.6 \pm 14.6\%$) was not significantly different from the control. The level of germination in the control was lower than levels achieved in previous trials using the same batch of seed, however, no hormones (GA₃ or zeatin) were used in the nutrient media for this trial.

4.4 DISCUSSION

In situ smoking of habitat soil stimulated the germination of *Boronia megastigma* seed in the soil but it did not stimulate the germination of all the seed in the seedbank, based on the number of germinable seeds in the soil determined from soil cores. This confirms the estimate of Plummer and Haddon (pers. observation) that the level of emergence after a fire only represents 1-2% germination based on estimates of annual seed production. Roche *et al.* (1997) found that smoke enhanced the germination response of seed stored in the soil for 1-2 years (see below). The profuse germination response observed following fire may be

stimulated by smoke but other factors (particularly ageing in the soil) are also required to stimulate germination in this species.

Seed collected from the soil germinated readily. The age of this soil-stored seed is unknown but it has to have been in the soil for at least 3 months (dehiscence is in December/January and the cores were taken in May). All the seed collected from the soil was highly scarified in appearance, suggesting that soil storage weakens the seed coat. It would seem that some form of seed ageing (possibly scarification) is required before the seed will germinate. These results confirm the findings of Roche *et al.* (1997).

Roche *et al.* (1997) found that *B. megastigma* seed (one year old) germinated when aged in the soil for 1 year ($1.1 \pm 0.6\%$) and 2 years ($2.5 \pm 1.4\%$) and this was enhanced when the seed was treated with aerosol smoke ($5.2 \pm 0.7\%$ year 1, $12.2 \pm 1.8\%$ year 2). Storage of seed in the soil for one year coincided with a decline in seed viability from 93% to 45%. A range of rapid ageing methods (nicking, leaching, acid scarification and wet-dry cycles), applied alone and together with smoke treatment, failed to simulate the germination achieved in soil stored seed. Soil storage in combination with smoke treatment is the key to achieving germination in this species when the seed is old (stored for 1 year or more after collection), however, these difficulties are not experienced in fresh seed.

Seed that is stored *ex situ* for more than a year shows no germination response even if the embryos are extracted and grown *in vitro* with growth hormones. Plummer *et al.* (in review), proposed that this germination decline is due to the development of an inhibitor after maturation which causes embryo dormancy. The endosperm was found to shrink during the final stage of seed maturation, coinciding with the onset of dormancy. Seed dehydration is often associated with increased endogenous abscisic acid, which is known to cause embryo dormancy in some species (Hilhorst and Karrsen, 1992). In the present study the rapid decline in germinability of seed harvested prior to dehiscence was also associated with a marked shrivelling of the endosperm. This shrivelling was not observed in seed harvested after dehiscence and may occur more rapidly in immature seed.

Plummer *et al.* (in review) found that *B. megastigma* had to be harvested prior to dehiscence as very few viable embryos were present in seeds collected at the final maturation date (87

days past anthesis). By comparison, this study found that maximum germination in wild-picked seed was achieved in seed collected after dehiscence ($78.8 \pm 7.8\%$) (this level of germination was only achieved in seeds which had their seedcoat nicked). The decline in germinability was less rapid than in seed collected prior to dehiscence but was still significant after two months storage. This would indicate that the rapid decline in germinability observed in pre-dehisced seed was due to a lack of maturity. The decline in viability in dehisced seed may be due to the accumulation of inhibitors.

Plummer *et al.* (in review) found that removing the seedcoat improved germination (max. 59.4%) for seed collected 82 DPA whilst excised embryos yielded 84% germination. In this present study it was found that nicking the seed coat achieved up to 96.7% germination in glasshouse plants (prior to dehiscence) and 78.8% germination in dehisced wild-picked seed. Seed collected from glasshouse plants had a higher viability than wild-picked seed. This is probably because the plants were grown under more optimal conditions. For the purposes of plant propagation, it is probably sufficient to simply nick the seed coat, a much simpler operation than embryo extraction and seedcoat removal.

Germination *in vitro* was only achieved in seed which had the seedcoat nicked in this study. There are three reasons why this may be required for germination to occur: (a) the seedcoat is a barrier to the imbibition of water; (b) the seedcoat is a barrier to the outward diffusion of endogenous germination inhibitors; and (c) the seedcoat is a barrier to the radicle emergence. Whole seed was found to absorb water readily (increasing in weight by 79.9% over one week soaking in water), so the seedcoat does not appear to be a barrier to the uptake of water. This would normally be caused by suberized and lignified cells in the testa which prevent water uptake. The benefits of soil storage in promoting seedling germination, and the scarified appearance of soil-stored seed, suggest that storage in the soil may assist in weakening the seedcoat to allow the inhibitor to leach from the seed or, alternatively, to allow the radicle to break through the seed coat.

Cryostorage was investigated as a means of halting the onset of inhibitors or viability decline in fresh-picked seed. Fresh seed of *B. megastigma* has a high moisture content (17.5%) which is not applicable to cryostorage. By desiccating the seed the moisture content of the seed can be reduced to a level (<15%) which enables it to be successfully frozen in liquid nitrogen.

Desiccation was successfully achieved using both LiCl_2 and a cryoprotective solution. Seed desiccated and then stored in liquid nitrogen for 2 days had the same germination success ($50.0 \pm 13.4\%$) as untreated seed ($50.0 \pm 10.0\%$). Although the germination success of seed which was not dehydrated prior to freezing was not significantly different from desiccated seed, the results of the first trial indicate the moisture content of the fresh seed is too high to be directly placed in cryostorage. Cryopreservation provides an effective means of halting germinability decline in this species, allowing seed to be stored indefinitely.

Based on the findings of this study, *B. megastigma* can be effectively propagated from seed. By placing stocking material over fruits prior to dehiscence, mature seed can be collected. This seed can either be used directly (stored in a fridge for 1-2 months) or placed in cryostorage for long term use. For cryostorage the seed should first be desiccated to a level below 15% moisture content and then plunged into liquid nitrogen storage indefinitely.

To germinate the fresh (or cryopreserved) seed it should be surface sterilized then nicked under sterile conditions and germinated in tissue culture on a media of 1/2 MS with GA3 and zeatin as described in section 4.2.3. In this way a high level of seedlings can be generated to assist in the development of new varieties of the species.

SECTION 2.

REFINING METHODS FOR SMOKE APPLICATION FOR NURSERY PROPAGATION AND FIELD SOWING.

Research Team Members

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CHAPTER 5. METHODS FOR APPLYING SMOKE FOR GERMINATION

5.1 INTRODUCTION

The phenomenon of smoke-stimulation of germination in Australian seeds has now been established for a broad suite of species. The natural progression is to develop commercially viable and effective methods to treat difficult to germinate seeds of species which have economic potential. Various methods have been developed to treat seeds with smoke. De Lange and Boucher (1990) are credited with first using aerosol and aqueous smoke to initiate seed germination using South African flora. In Australian species, both smoked water and aerosol smoke have been shown to improve germination (Dixon *et al.*, 1990; Roche, 1993). Three effective methods are now predominantly used: (a) Application of aerosol smoke to seed trays; (b) Direct application of aerosol smoke to seed; and (c) Application of smoke water to seed trays and smoke water imbibition of seed.

Each of these methods is described in detail below, including experiments to refine the techniques and the benefits and disadvantages of each form of application.

5.1.1 Aerosol smoke

Seeds are sown in punnets and placed into a sealed, steel-framed plastic tent measuring 2x1.5x1m. Smoke is generated in a 60-litre steel drum by slow combustion of organic materials (Fig 5.1). The type of plant material from which smoke is derived is not particularly important in generating the final product however generally a mixture of fresh and dry native plant leaf and stems is used. The drum is fitted with an inlet through which air is pumped at the rate of 30-50L/min, and the resulting smoke is cooled as it passes through a 2m length of flexible plastic hosing before passing into the fumigation tent containing the seeds sown in punnets. Using this method, the air temperature in the tent rises by 4°C over 1 hour, and the temperature of the soil placed within it is not appreciably increased (Roche, unpublished data). The tent can contain up to three levels of shelving. Approximately 30cm should be left between each shelf to ensure adequate flow of smoke between shelves. After 60 minutes in

the smoke tent, punnets are transferred to the glasshouse and gently handwatered for the first week to ensure the smoke chemical is not washed out of the punnets. Smoked soil surfaces emit a distinctive smoked odour which lasts for 6-8 days.

Research conducted at Kings Park and Botanic Garden, using a wide range of species, has found 60 minutes to be the optimum exposure time for aerosol smoke treatment (Dixon *et al.*, 1995; Roche 1995a). Thirty min and less seem to limit germination for some species and durations longer than 60 min (90 min) appear to be inhibitory (Roche, 1993).

Germination using aerosol smoke has been shown to be higher than other methods of smoke application (Roche, 1993; Dixon *et al.*, 1995). For this reason it is a highly effective means of treating seed sown in punnets for nursery use. Unfortunately the method does not readily lend itself to the broadacre situation. The method has other limitations as well. The smoke tent is time consuming to set up and operate and poses health hazards for people involved. Seed treated in this way cannot be stored for later use or directly sown in the field.

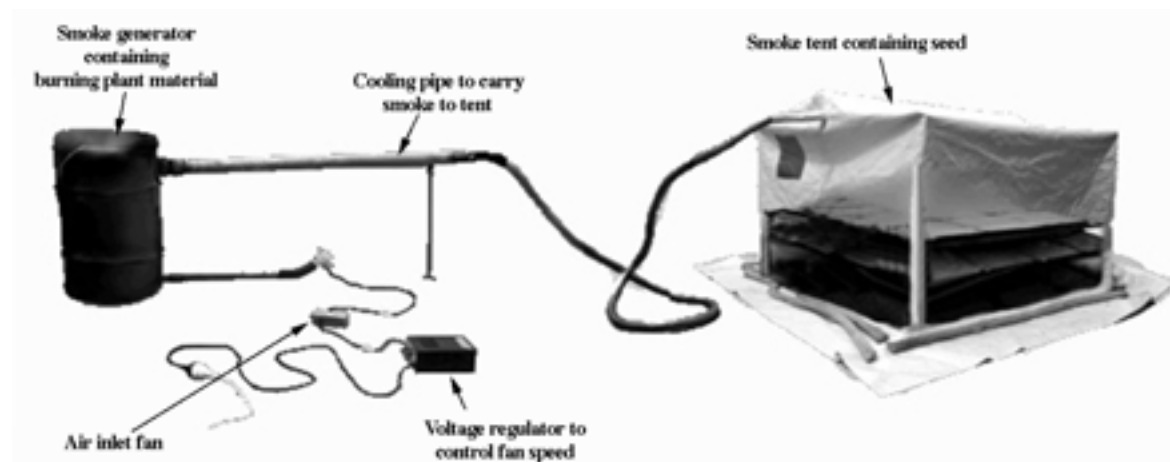


Fig. 5.16 Apparatus for aerosol smoke generation.

5.1.2 Direct application of aerosol smoke to seed

Aerosol smoke, generated using the smoke tent method (described above), can also be directly applied to seed. The general method now used is to place the seed on an open tray in a smoke tent for one hour. The seed can then be either stored indefinitely or sowed directly.

In a study by Roche *et al.* (1997) it was shown that the direct application of aerosol smoke to broadcast seed produced significant increases in the total numbers of germinants and species in a rehabilitated mine pit. Total numbers of germinants was increased by over 85% while there was a 34% increase in the species richness compared with untreated broadcast seed. This method of smoke application is now widely used by mining companies to treat the broadcast seed used in rehabilitation.

Seeds treated with aerosol smoke become covered with sticky smoke residues (Roche, 1993). In some cases this residue makes the seed unsuitable for mechanised sowing and unpleasant to handle for home gardeners. This problem arises in the treatment of some fine seed and is a limitation of this method.

5.1.3 Aqueous smoke

Preparation of aqueous smoke has greater commercial potential for broad scale application than aerosol smoke. Attempts to commercialise smoke as a germination tool has generally focused on aqueous smoke instead of aerosol smoke to treat seeds due to a number of key benefits. The concentration of aqueous smoke can be controlled - an issue of special importance to smoke-sensitive species (Brown *et al.* 1993a; Dixon *et al.* 1995). Aqueous smoke is much easier to apply, being suitable for mechanical sowing. In addition, the smoke cue can be retained in the seed during storage (Baxter and Van Staden, 1994).

Smoked water is produced by bubbling smoke from a 60-litre steel combustion drum through 100 litres of water for 60 minutes. A mix of fresh and dry native plant cuttings are used as fuel (Fig. 5.2). A sample from each new batch of aqueous smoke can then be tested on a bioassay species (*Stylidium affine*) to ensure it elicits the germination promoting response (see section 1.4).

Part of this work has focused on determining if increasing the concentration of combustion products in water will enhance germination. Smoke was bubbled through water for 10, 20, 30, 40, 50 and 60 minutes and samples diluted to produce 1/100, 1/10, 1/5, 1/2 and undiluted solutions of smoked water. In *S. affine* optimal germination response (50 %-60 %) was recorded at the 1/10 dilution for all smoking periods, however similar germination responses

may be obtained when water is smoked for a shorter period of time (10 & 20 minutes) at 1/2 strength. This result clearly indicates that the germination enhancing factor(s) rapidly saturates the aqueous solution at 10 minutes.

The optimum concentration of smoke water for germination is generally 10% of full strength. This was determined using the indicator species *S. affine*. Seed was germinated on filter paper moistened with various concentrations of smoked water (1/100, 1/10, 1/5, 1/2, undiluted smoke water and deionised water) and incubated at 19° C for seven weeks. Maximum germination of 60 % to 70 % was obtained at intermediate concentrations (1/10, 1/5, 1/2), as compared to 50% germination for the undiluted and 1/100 solutions (Fig. 5.3). Germination was earlier at the lower concentrations than the undiluted solution. Seed on filter papers moistened with deionised water did not germinate.

Generally seeds are imbibed in 10% smoke water for 3-6 hours for best results. This optimal exposure period at 1/10 dilution of smoke water was determined by imbibing seed of *Styloidium affine*, *Actinotus leucocephalus* and *Geleznovia verrucosa* in an aerated solution of smoke water or deionised water for periods of 0, 3, 6, 12, 18, 24 and 48 hours (Fig. 5.4). Imbibed seed was weighed to determine the rate of water uptake by seed. Seed was sterilised and placed onto filter paper moistened with deionised water and incubated for seven weeks. Seed imbibed rapidly between one and three hours with a levelling of the dose response at 18 hours. Two optimal response periods were recorded for *S. affine* and *A. leucocephalus* with maximum germination (20%) occurring at three and six hours and again at 48 hours. Germination of *G. verrucosa* was uniform across all exposure periods. Seed of each of the three species did not germinate when imbibed in water alone.

Once prepared, smoke water can be used immediately without specific equipment and is storable, when frozen, for at least 2 years.

Aqueous smoke is the most commercially adaptable smoke treatment, having many practical benefits over aerosol smoke. Some species, such as *Conostylis* species (Roche, 1993), germinate just as well from aerosol and aqueous smoke, however, aerosol smoke achieves greater results.

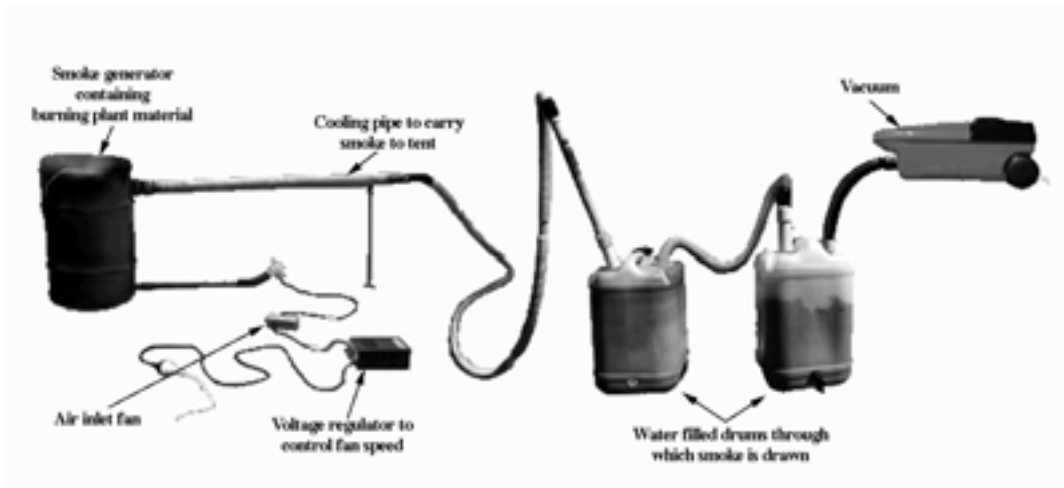


Fig. 5.26 Apparatus for aqueous smoke generation.

Research continues to improve the effectiveness of aqueous smoke treatment and to develop its commercial application. An important step will be the development of a method to prime seeds with smoke prior to storage. It has been found that SW imbibed seeds that are stored retain the germination trigger of smoke. In the grass species, *Themeda triandra* Forssk. The optimum imbibition duration for seed germination was 12 hours after storage for 21 days (Baxter and Van Staden, 1994). Germination was found to be even higher after dehydration and storage. If seeds of Australian species imbibed in smoked water retain high germinability after a period of storage this method may be established as an effective means of pretreating seed.

CHAPTER 6. DEVELOPMENT OF DIRECT IMBIBITION, OSMOCONDITIONING AND INFUSION METHODS FOR PRE-PRIMING SEEDS WITH AQUEOUS SUSPENSIONS OF SMOKE.

6.1 INTRODUCTION

Various procedures that initiate germination prior to planting are used to improve the speed and uniformity of seedling establishment and to overcome various dormancy problems in freshly harvested seed. These methods are called seed priming (Armitage, 1994). Three such methods, pre-imbibition, osmoconditioning and infusion, were investigated in this study with

the aim of determining if these methods can facilitate the uptake of smoke water into native seeds and provide uniformity in germination as a prerequisite for automated sowing.

The most simple means of seed priming is to imbibe seed in a solution and then allow the seed to dry. This reduces the time required for germination and allows chemicals to be incorporated into the seeds (Khan *et al.* 1978). Pre-imbibition of seed, also referred to as priming is widely practised in commercial agriculture and horticulture to promote fast and uniform germination.

Osmoconditioning involves treating seed with an external high osmotic solution to decrease the osmotic potential within the seed. Metabolic activities leading to germination proceed but the emergence of the radicle is prevented or delayed. Osmoconditioning is widely used within broadscale horticulture to initiate germination before planting to reduce time to germination, and to improve uniformity of the germination rate. The inert compound polyethylene glycol (PEG 6000) is most often used, but salt solutions of NaCl and KNO₃ are occasionally used.

Organic solvent infusion is a method for incorporating chemicals, such as growth regulators, into seeds by means of organic solvents. Seeds are immersed for one to four hours in an acetone and dichloromethane solution which contains the chemicals to be infused. The solvent is then removed by evaporation and the seeds are dried in a desiccator. The incorporated chemical is then uptaken directly into the embryo following imbibition in water.

For smoke pretreated seeds to be of commercial value, they need to retain the activation compound of smoke after a period of storage so that dry seed can be sown. Baxter and van Staden (1994) investigated the capacity of smoked water treated seed of the South African grass species, *Themeda triandra* to retain the active smoke compound. They found the promotive effect of smoke remained after drying and storage for three weeks. Germination was even enhanced after dehydration and storage. The study was concerned that seedling growth would be adversely affected after seeds were pretreated with aqueous smoke, however, it was found that pretreated seeds produced seedlings with 'normal' growth and development (Baxter and van Staden, 1994).

The aim of this chapter is to investigate seed priming techniques for Australian native seed. Australian species imbibed with aqueous smoke (derived from combustion of plant material) were investigated to determine whether the promotive effect of smoke on seed germination is retained after seed was dried and stored. Pretreatment of Australian native seed with polyethylene glycol and smoked water solution was investigated to determine if it would result in a decrease in the rate of germination and an improvement in the uniformity of germination. The use of organic solvents to enhance the infusion of smoke water into native seed was investigated using acetone as a solvent.

6.2 METHODS AND MATERIALS

6.2.1 Smoke water imbibition experiment

The study species *Conostylis setigera*, *Actinotus leucocephalus* and *Stylidium brunonianum* were used in this first experiment. Previous studies have shown that smoke water dramatically enhances the germination of these species (Tieu, et al. 1998, in press).

Three replicates of seed (n=50) for each time treatment were enclosed in a square nylon sachet (2 cm²). Due to their small size, *S. brunonianum* seeds were wrapped in 4 cm filter paper (No. 1, Whatman). Smoked water was prepared as described in Chapter 1 and seeds were imbibed for 0, 3, 12, 24 or 48 h in 10 % smoke water (*C. setigera*) or 20 % smoke water (*A. leucocephalus*, *S. brunonianum* and *S. affine*) or distilled water (based on Tieu, *unpubl.*). Smoked water solutions were aerated (with a household aquarium pump) and after different treatment periods, imbibed seeds were then transferred to a laminar flow for surface sterilisation.

Seeds were soaked and agitated in a solution of hypochlorite for 2 min and rinsed with 2L distilled water. Sachets were dried by pressing between paper towels, seeds removed and placed onto 2 layers of sterilised filter paper (No. 1, Whatman) in Petri dishes (10 cm) with 1.5 ml Benlate solution® (0.04 g/L) to prevent fungal contamination. To compare imbibed smoke water treatments of seeds and those with continuous exposure of smoke water, three replicates of each species were incubated on 20 % smoke water except for *C. setigera* which

was 10 % smoke water, on filter paper in Petri dishes. Seeds in this experiment were sterilised as outlined as above.

Seeds for the drying treatment were soaked in the same concentration and duration of smoked water pretreatment as described above. Instead of plating directly, following imbibition, seeds were dehydrated for 24 h in a laminar flow with a constant air flow (4 ms^{-1}), and then sterilised and plated as described above. Seeds that were to be stored were dehydrated for 24 h, when seeds were dry and flowing in the laminar flow, stored for 3 weeks in air tight containers at room temperature and then disinfested before incubation.

All petri dishes were sealed with plastic wrap to prevent contamination and moisture loss. Plates were arranged in a randomised complete block design in light-exclusion containers in an unlit incubator at 19°C . Germination was scored when there was the first sign of radicle emergence with scoring done weekly from the 3rd to 7th week.

Statistical analysis

Germination results were transformed to percentages and adjusted for viability by multiplying raw data by viability of each species. Least significant differences (LSD) between the control and smoked water treatments at 5% confidence level was determined by Analysis of Variance (ANOVA). Data that were not normally distributed were transformed by Angular Arcsine Transformation and their ANOVA's performed.

6.2.3 Osmoconditioning experiment

Species Selection

Two species were selected for the development of osmoconditioning and infusion pre-treatments: *Conostylis candicans*, a species that has its germination enhanced by smoke water treatment; and the bioindicator *Stylidium affine*, a species that will only germinate after treatment with smoke water (no germination in water controls).

Seed of these species was counted into lots of 100, 3 replicates per treatment, and sealed in nylon sachets (50mm x 50mm). The plating method used in these experiments did not require that the seed be surface sterilized.

Full strength smoked water was prepared using the procedure described in section 1.3.2 and then diluted with deionized water to produce 1/5 and 1/10 concentrations of smoked water.

Osmoconditioning

Solutions of 20% and 30% polyethylene glycol (PEG 6000) alone were prepared by adding PEG 6000 crystals to deionized water and stirring with a magnetic stirrer until all crystals were dissolved. Combined smoked water and PEG solutions were prepared by adding PEG 6000 (200g or 300g) to prepared smoke water solutions to produce the following solutions:

Control (water)	1/10 smoke water + 20% PEG
smoke water 1/10	1/10 smoke water + 30% PEG
smoke water 1/5	1/5 smoke water + 20% PEG
PEG 20%	1/5 smoke water + 30% PEG
PEG 30%	

Labelled seed sachets (3 replicates per treatment) were immersed in aqueous smoke and PEG solutions (300ml) for 7, 14 or 21 days. The experiment commenced with the 21 day treatment, with all seed sachets removed from the solutions, simultaneously.

After 21 days, solutions were discarded and seeds washed in three to five washes of distilled water, or until all evidence of the PEG solution was removed. Sachets were dried to remove excess moisture and placed in an incubator at 25°C for 24 hours. Dried seed was removed from nylon sachets, placed into seed envelopes and stored for two weeks, under laboratory conditions.

Seed Sowing:

Sterilized white sand (100g) was spread onto a 5mm layer of water agar in sterile 250ml containers. Unsterilized seed was spread evenly over the sand and covered with a layer of

sterilized white sand (50g). Seed was incubated in the light at 19°C ($\pm 2^\circ\text{C}$), and the numbers of germinants recorded weekly.

Data analysis

The number of germinants were scored weekly. Germination performance was analyzed using the following equation:

$$k=0.693/(t_{50}-t_0),$$

where t_0 is the number of germinants in the first week scored and t_{50} is the time required to achieve 50% maximum germination (Milthorpe and Moorby 1974). The parameter k is a measure of the variation in time required for a seed to germinate in the population. The data was analyzed by 3 factor ANOVA (PEG concentration x smoke water concentration x imbibition time).

6.2.3 Acetone infusion experiment

Preparation of treatment solutions

Three 1L treatment solutions were prepared: acetone with smoke water; smoke water alone and acetone alone. Acetone solutions were prepared with full strength smoked water to produce concentrations of 1/10 smoke water (100ml of smoke water added to 900ml of acetone).

Sachets of seed were immersed in one of the three treatment solutions (3 replicates per treatment) for 1, 2 or 4 hours, commencing with the four hour treatment. After four hours, seed was removed simultaneously and placed under vacuum for two hours to remove the acetone. After vacuum treatment, seed was removed from nylon sachets and stored in paper seed envelopes for two weeks at 25°C.

Germination

Seed was sown in 250ml containers with agar and sterile sand as described for the osmoconditioning experiment above. Replicates of each treatment were stacked in an

illuminated incubator at a constant temperature of $19^{\circ}\text{C}\pm 2^{\circ}\text{C}$, and monitored weekly for a period of 12 weeks.

6.3 RESULTS

6.3.1 Smoke water imbibition experiment

Seed imbibed in smoked water was effective in enhancing germination for all test species. Germination of smoke water imbibed seeds that were incubated without a drying interval, were equal or higher to those in continuous contact with smoke water for *C. setigera*, *S. brunonianum* and *A. leucocephalus* (Fig. 6.1). Germination of *S. affine* was significantly lower by only 10%. smoke water imbibed seeds (dehydrated and consequently stored), retained the activating smoke cue and germinated as successfully as seeds that were in continuous contact with smoke water for *C. setigera*, *S. brunonianum*, *A. leucocephalus* and *S. affine* (Fig. 6.1).

Conostylis setigera

The efficacy of smoke water imbibition of *C. setigera* seed were parallel to those in continuous contact with smoke water (Fig. 6.1). Lower duration of imbibition (3 and 6 h) were the most effective in enhancing germination of seeds plated immediately after imbibition (Fig. 6.1). Seeds that were dehydrated after being imbibed for 3 and 6 h gave lower germination compared to those plated directly (Fig. 6.1). Germination rates increased with increasing period of imbibition after dehydration as 24 and 48 h were as high as those in continual contact with smoke water. This trend was also seen in dehydrated and stored seeds (Fig. 6.1).

Stylidium brunonianum

Germination of imbibed *S. brunonianum* seeds, incubated immediately were similar to untreated seeds plated on smoked water (Fig. 6.1). The optimal duration of imbibition was 24 h but this was not significantly different to 6, 12 or 48 h (Fig. 6.1). Dehydration of imbibed seeds lowered germination (Fig. 6.1) but those subsequently stored, were as high as those

incubated immediately (Fig. 6.1). The optimal duration of dehydrated and stored seeds was 12 h (Fig. 6.1).

Actinotus leucocephalus

Imbibition of *A. leucocephalus* seeds in smoked water was more effective than seeds exposed to smoke water during 7 weeks of incubation (Fig. 6.1). Dehydrated and stored seeds germinated as successfully as those plated immediately after imbibition. Optimal germination of dehydrated as well as stored seeds favoured longer duration (Fig. 6.1). After dehydration, seeds required at least 24 h for germination to occur (Fig. 6.1) but only 6 h if dehydrated and stored (Fig. 6.1).

Stylidium affine

Germination of *S. affine* seeds imbibed in smoke water and plated immediately were significantly lower than those in continuous contact with smoke water (Fig. 6.1). No clear optimal duration of imbibition could be observed for seeds incubated immediately, nor those that were dehydrated. However, imbibition for 3, 6 and 12 h gave significantly higher germination of dehydrated and stored seeds than if they were imbibed for 24 or 48 h (Fig. 6.1). Germination in dehydrated and stored seeds (Fig. 6.1) and were the same or higher than seeds plated immediately and dehydrated after imbibition (Fig. 6.1).

6.3.2 Osmoconditioning experiment

The uniformity of germination was reduced in *Conostylis candicans* ($p < .005$, df 2, Fig. 6.2a) and there was no improvement in uniformity in *Stylidium affine* with the addition of PEG ($p > .05$, df 1, Fig. 6.2b). The addition of PEG did not affect the time of germination in *Conostylis candicans*. Germination at t_0 and t_{50} ($p > .05$, df 2). *Stylidium affine*, however, germinated earlier with the addition of higher PEG concentrations. Germination at t_0 increased from $16 \pm 2.28\%$ in the control to $22.75 \pm 1.44\%$ at the 30% PEG concentration ($p < .01$, df 2, Fig. 6.3). The interaction of smoke water concentration and PEG concentrations did not show any improvement in the uniformity of germination (k) for either species.

6.3.3 Acetone infusion experiment

There was no significant improvement in germination as a result of acetone treatment. Seed of *Stylidium affine* germinated more readily when treated with smoke water alone ($x=36.7\pm 10.8$) compared with smoke water with acetone ($x=11.7\pm 1.6$) ($P=0.02$) (Fig. 6.4a). There was no significant difference between treatments for *Conostylis candidans* ($p=.12$) (Fig. 6.4b).

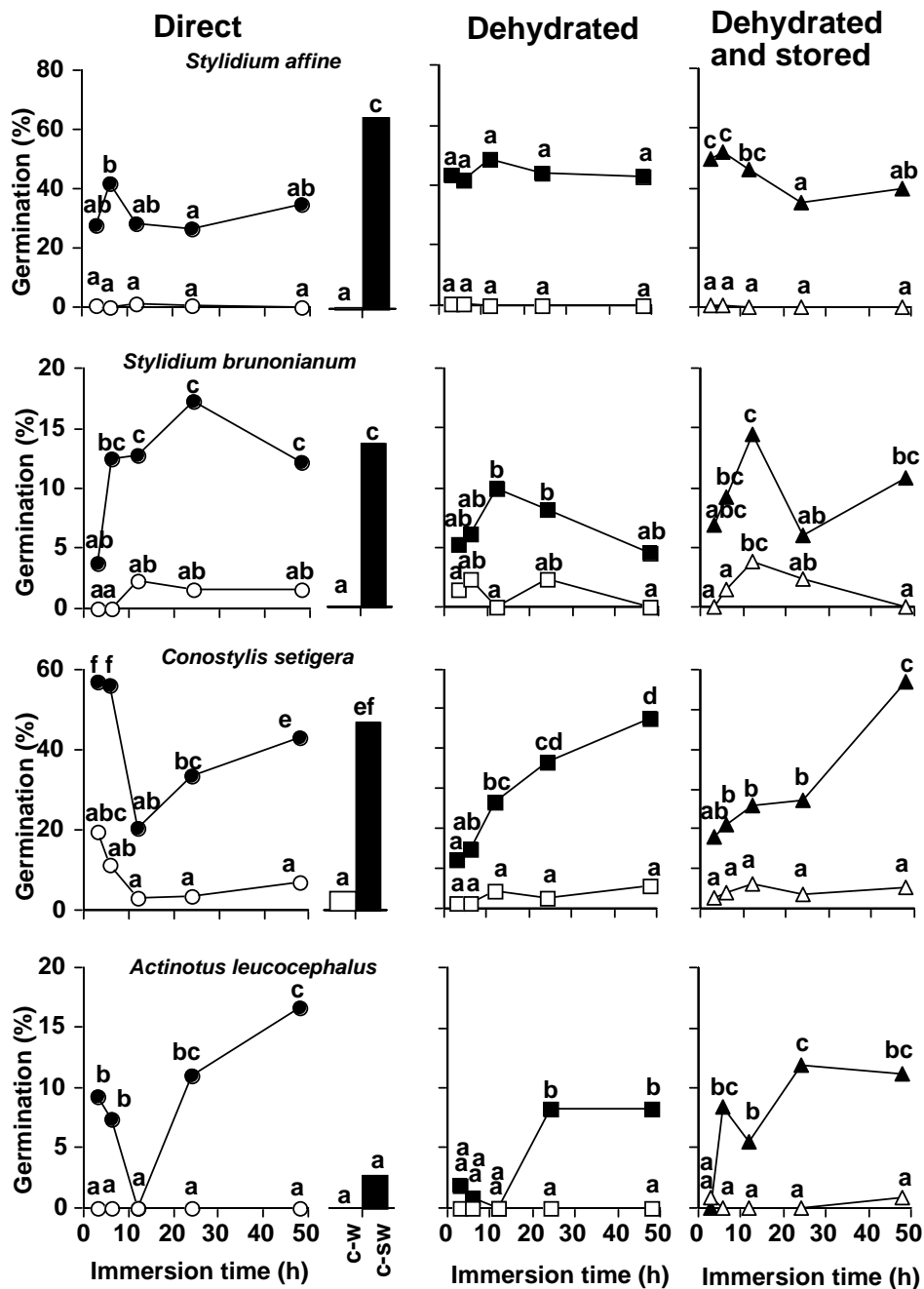


Fig. 6.1—Germination (%) of *Stylidium affine*, *Stylidium brunonianum*, *Conostylis setigera* and *Actinotus leucocephalus* after 7 weeks, incubated on water immediately (ll), after 24 h dehydration (nn) and after 24 h dehydration and 3 weeks storage (ss) following immersion for 3, 6, 12, 24 or 48 h in water (open symbols) or 20 % smoke water for all species (except for *C. setigera*; 10%) (solid symbols). Bar graphs indicate continuous contact of seed with water (c-w) or smoke water (c-sw). Values followed by the same letter are not significantly different ($P < 0.05$) (Calculated LSD compared water (continuous, lns) and smoke water (continuous, lns) treatments separately).

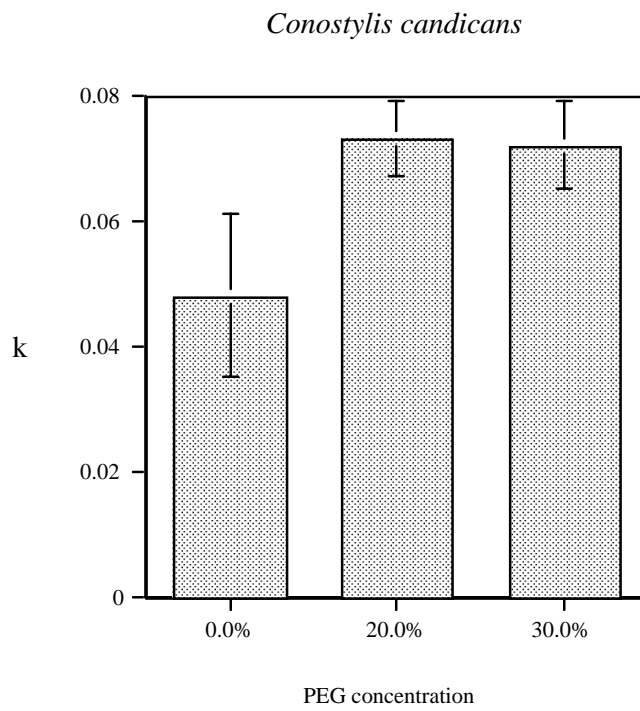
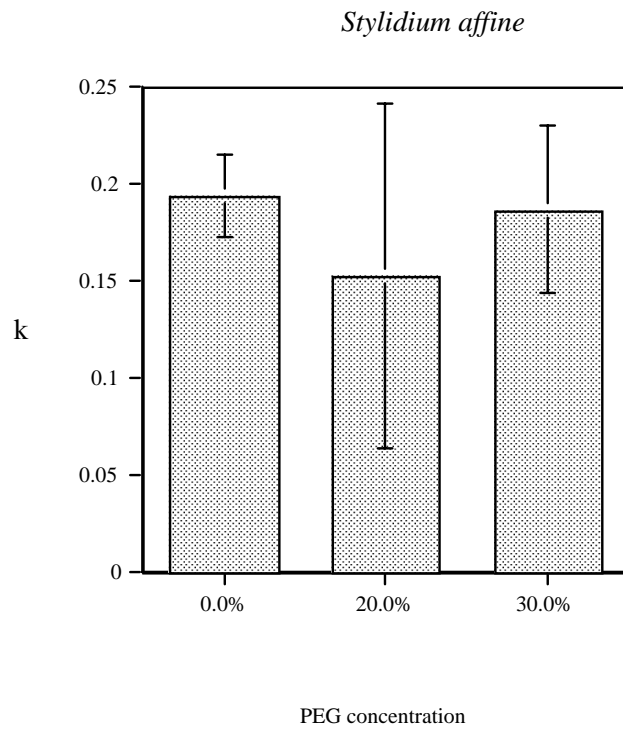


Fig. 6.2—The uniformity of germination (k) of *Stylidium affine* (a) and *Conostylis candicans* (b) in three treatments of PEG (0, 20, 30%).

Stylidium affine

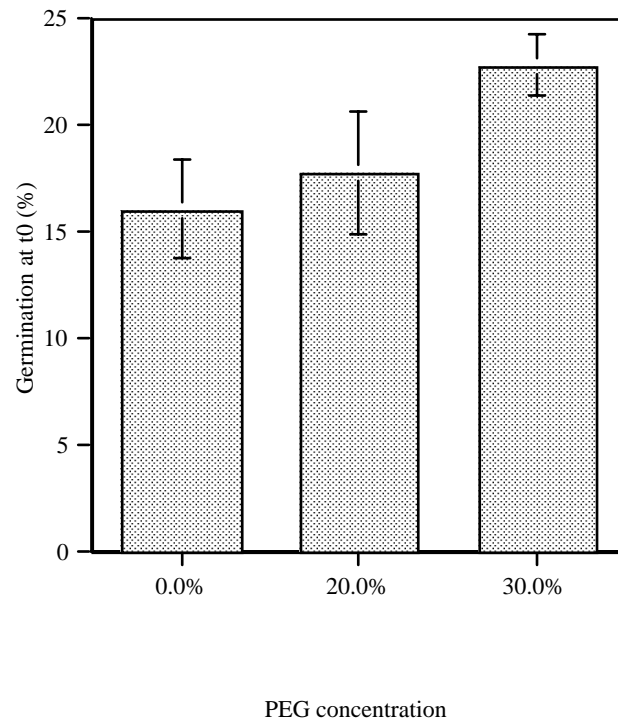


Fig. 6.3—The level of germination at the time of the first count (t0) of *Stylidium affine* in three treatments of PEG (0, 20, 30%).

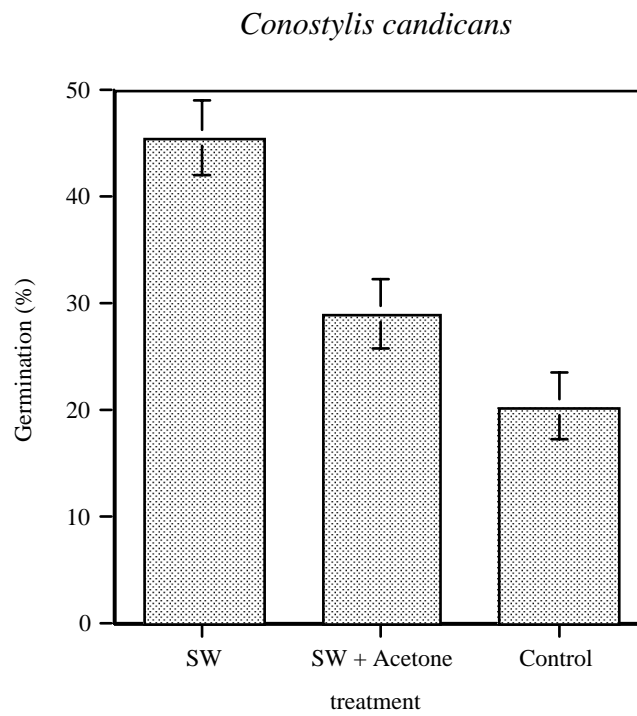
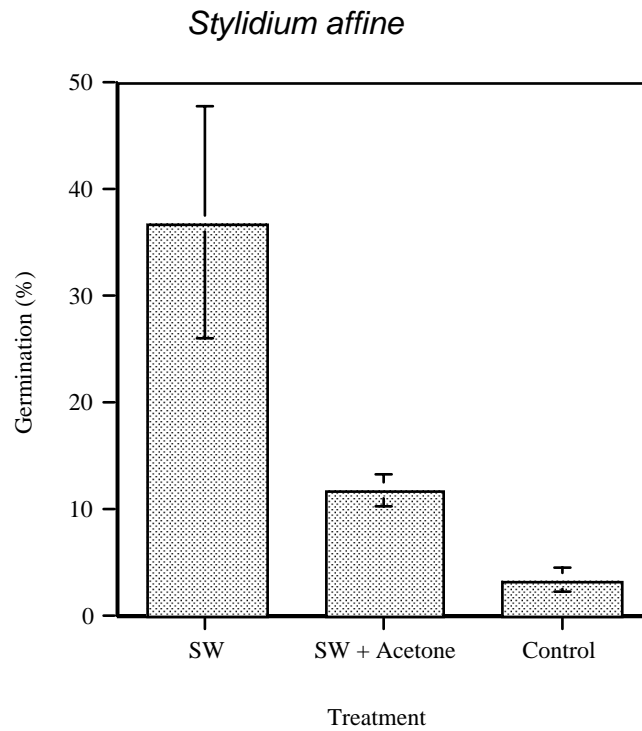


Fig. 6.4—The percentage germination of *Stylidium affine* (a) and *Conostylis candicans* (b) treated with acetone and smoke water.

6.4 DISCUSSION

Imbibition in smoked water was effective in enhancing seed germination of *C. setigera*, *S. brunonianum*, *A. leucocephalus* and *S. affine*. Germination was comparable to those seeds in continuous contact with smoked water. The effectiveness of smoke water imbibition was also observed by Baxter and Van Staden (1994) with *Themeda triandra* when germination of imbibed seeds (24 h) increased to 25.9 % compared to 9.1 % in the control. The efficacy of smoked water imbibition was retained after dehydration and storage and was highest when seeds were imbibed for longer periods (24 and 48 h) for *C. setigera* and *A. leucocephalus*.

Differences in optimum duration of imbibition for the four species could be explained by inherent differences that exists between each species. This could affect the rate of entry, permeability and sensitivity of seed tissues to active compounds in smoke water. However, *C. setigera* showed that undehydrated seeds favoured lower imbibition durations (3 and 6 h) and dehydrated and stored seeds, higher durations of imbibitions (Fig. 6.1). Seeds probably failed to germinate well after longer durations of imbibition as excessive water availability may have damaged seed tissues (Leopold and Kriedemann, 1975).

Dehydration of smoked water imbibed seeds on the other hand, required longer periods of imbibition which is possibly due to reduced rates of water uptake rates. Prior to dehydration, imbibition could have initiated seed metabolism, thus reducing sugar concentrations through increased respiration which leads to lower osmotic potential (Bewley and Black, 1995). Therefore when reimbibed, water uptake will be slower. Additionally, upon reimbibition, hydrolysed sugar molecules could have been leached and further reduce the osmotic potential of the seed. Imbibition duration of dehydrated seeds would therefore have to be increased, to compensate for lower rate of water uptake (Fig. 6.1).

Seeds imbibed in smoke water, dried and stored for 3 weeks retained the activating smoke compound as percentage germination were comparable to seeds that were not stored (Figs. 6.1). *S. affine*'s germination percentage was found to be 5 % higher when treated seeds were dried and stored compared to seeds plated immediately (Fig. 6.1). This trend was also observed by Baxter and Van Staden (1994) who found that the smoke cue in pretreated seeds

were viable after storage and the processes leading up to storage further enhanced germination of *Themeda triandra*, from 25.9 to 36.6 %.

The ability of stored seeds to retain the active smoke compounds demonstrates that the smoke cue seems to be a stable compound and is not lost from or inactivated within the seed after drying or storage. It appears that the compound is either absorbed or adsorbed and remains persistent until conditions are conducive to germination. Baldwin *et al.* (1994) in discussing the stability of the smoke cue, showed that the smoke cue remained active for at least 53 days in soil under greenhouse conditions. The effectiveness of relatively short periods of imbibition (3 h), also demonstrates that low exposure time to smoked water is adequate to trigger seed germination. Again, this characteristic of smoke water was observed by Baldwin *et al.* (1994).

As it was found that the most effective imbibition duration for imbibed seeds 'dehydrated only' were the same as those 'dehydrated and stored', it is likely that the slightly lower germination of *S. brunonianum* and *A. leucocephalus* and also higher germination of *S. affine* after storage was due to the act of drying and not storage.

PEG was not an effective agent for osmoconditioning in *Conostylis candidans* or *Stylidium affine*. There was no evidence to suggest it enhanced the imbibition of smoke chemicals into the seed or improved the uniformity of germination in these study species. PEG did have a positive effect in increasing the initial rate of germination in *Stylidium affine*, however this effect was not significant enough to warrant further investigation. It is possible that in the species investigated in this study the differences in vigour between seeds in each lot were too great for them to be effectively compensated for by PEG to allow uniform germination. The use of osmoconditioning agents does not appear to be effective for the species studied however, considering the diversity of imbibition and germination responses in Australian native seeds, a much wider range of species needs to be investigated and other osmoconditioning agents may need to be pursued.

The use of acetone as an organic solvent for infusing the smoke water solution into seeds was also not successful at enhancing the germination rate or the uniformity of germination. There may be a number of reasons for this poor response. It may be that the active agents in smoke

water and acetone do not effectively mix due to differences in polarity. Acetone generally works by removing some of the waxes from the seed coat thus speeding up imbibition into the seed. Egerton-Warburton (1997, unpubl.) found aqueous water also acted by improving the permeability of the seed coat. It would seem from these results that acetone does not enhance the uptake of smoke water into the seed any more efficiently than smoke water alone suggesting that waxy, impermeable layers are possibly not involved in smoke-mediated dormancy.

Based on the results of this study the pre-imbibition of smoke water alone appears to be the most efficient means of pretreating seed. This can be achieved by preimbibing seed in 10 or 20% smoke water for 12-48 hours, then air drying the seed for storage in an air-tight container.

CHAPTER 7. FINAL DISCUSSION

7.1 FULFILMENT OF KEY OBJECTIVES

Objective 1. Refine smoke stimulation of target species.

The mechanism by which smoke overcomes dormancy in Australian native seeds is still poorly understood. The results presented in Chapter 3 suggest that the dormancy factor is located in the seed coat in *Stylidium affine* whilst in *Actinotus leucocephalus* the inhibitory mechanism was located in the endosperm and/or embryo. Smoke water acted on different tissues in these species to overcome dormancy. The work on *Stylidium affine* suggests that smoke water acted by overcoming inhibitors in the seed coat, supporting the results of Baldwin *et al.* (1994) who proposed that smoke acts as a chemical functionary. The results are at odds with the findings of Egerton-Warburton (1997, in press) who found that dormancy in *Emmenanthe penduliflora* was imposed by the seed coat preventing the outward movement of inhibitors. Smoke was found to act on the sub-testa cuticle to allow the outward movement of inhibitors, relieving dormancy.

It can be concluded that smoke acts in a number of ways to overcome a variety of dormancy mechanisms present in seed of different smoke-responsive species. An understanding of how smoke breaks dormancy will be essential for future development of these techniques. The active ingredient of smoke and how it overcomes dormancy in such a wide range of species is still to be determined.

Objective 2. Devise methods for smoke stimulated germination of large seeded species, marginally germinable species and other species which have not responded to current smoke procedures.

With the discovery of smoke-mediated germination it is now possible to better understand the heterogeneity in germination patterns for a wide range of Australian plant species. In the study presented in Chapter 2 over 180 species were examined to determine why they are not initially responsive to smoke. The study examined soil storage and a number of commonly

used seed pre-treatments in combination with smoke to examine both longevity in artificially constructed seed banks, and the role of seed ageing in improved germination.

In general, species germinated maximally either in their first year or after soil storage. When fresh seed was used, almost 70% of species tested responded positively to smoke whether applied prior to or after sowing. Variation in success, however, confirmed earlier conclusions that substances contained in plant-derived smoke may be inhibitory at high concentrations for particularly sensitive species. Only 10% of species under investigation recorded optimum germination with seed ageing alone but when smoke was applied as a treatment after soil storage, 60% of species responded positively. The trade off with soil storage is the viability decline which occurs. Over 1 year, the viability decline in soil-stored seed of the range of species examined varied between 10 and 80%. Reductions of as little as 15% were found to compromise the ability of a number of species to successfully recruit in consecutive seasons. Rapid ageing was not as successful in eliciting a response, even when smoke was applied. Some exceptions to this rule were *Astroloma foliosum* (fruit removal before smoking), *Billardiera varifolia* (removal from pods before smoking), *Hibbertia commutata* (seed coat removal before smoking), *H. lasiopus* (seed coat removal before smoking), *H. sericea* (nicking before smoking), *Leucopogon capitellatus* (acid treatment before smoking), *Scaevola crassifolia* (acid treatment before smoking) and *S. fasciculata* (acid treatment before smoking).

Objective 3. Determine optimal smoke concentration of smoke fumigation exposure times for eliciting germination responses.

The optimal concentrations of smoke and aqueous smoke application for eliciting germination responses have now been determined. Aerosol smoking of seed and seed sown in punnets is 60 minutes in the smoke tent. Thirty minutes and less may limit the germination of some species and durations of longer than 60 minutes may be inhibitory.

The application of aqueous smoke (as described in section 5.1.3) has been found to be most effective at a concentration of 10-20% of full strength. The optimal imbibition time is 3-6 hours for most species.

Objective 4. Refine smoke generation apparatus and methods for smoke application i.e. granulation, liquid application.

The smoke generation apparatus of three main methods for smoke application have now been refined.

Aerosol smoke is generated in a 60-litre steel drum by slow combustion of organic materials. The type of plant material from which smoke is derived is not particularly important in generating the final product however generally a mixture of fresh and dry native plant leaf and stems is used. The drum is fitted with an inlet through which air is pumped at the rate of 30-50L/min, and the resulting smoke is cooled as it passes through a 2-m length of flexible plastic hosing before passing into the fumigation tent. Seeds, either sown in punnets or exposed directly are placed into the tent. After 60 minutes in the smoke tent the punnets or seeds are transferred to the glasshouse and gently handwatered for the first week to ensure the smoke chemical is not washed out of the punnets.

Smoked water is produced by bubbling smoke from a 60-litre steel combustion drum through 100 litres of water for 60 minutes. A mix of fresh and dry native plant cuttings are used as fuel. Twelve hundred litres of smoked water can be produced using this batch system. The optimum concentration of smoke water for germination is generally 10-20% of full strength. Seeds are imbibed for 3-6 hours or overnight. Once the smoke water is prepared it can be frozen and stored for up to 2 years.

Objective 5. Develop smoke application procedures for stimulation of germination in field and evaluate the best methods for subsequent establishment of plants.

For the purposes of field sowing there are two methods of smoke-priming seed for germination. Firstly, the broadcast seed can be exposed to aerosol smoke in a smoke tent for one hour (as described above in objective 4), coating the seed with a sticky residue. This method is effective for a wide range of species, and is widely used to treat broadcast seed used in minesite rehabilitation. The residue coating the seed can cause seed to stick together making it difficult to broadcast. This is particularly a problem with finer seed.

A second method is to prime the seed with aqueous smoke and then dehydrate it prior to broadcasting. This method is described in detail below.

Objective 6. Develop procedures for direct application of smoke or the germination enhancing principle to seed i.e. coatings, pre-imbibing or encapsulating technology.

The most proficient way of applying smoke treatments in the nursery and broadacre situation is through aqueous smoke.

If seeds are to be sown directly the general procedure, refined at Kings Park and Botanic Garden, is to imbibe the seed in 10-20% full strength smoke water (produced as described in section 5.1.3) for 3-6 hours or overnight.

If seeds are to be preimbibed prior to storage or broadcasting in the field they should be imbibed for a longer period (12-48 hours). Once imbibed the seed can then be air dried ready for storage or field sowing. Seed treated in this way can then be stored for at least 3 weeks and is smoke-primed for germination.

Objective 7. Develop a protocol for use of smoke germination technology in nursery propagation and possibly direct sowing of economically significant species.

Research conducted as part of this study has helped to refine smoke technology for commercial application. The protocol listed below describes a procedure for applying smoke technology to new species of plant for nursery propagation or broadacre use.

PROTOCOL

Developing species for nursery production

Step 1. Ensure the seed batch is mature and free of any granivorous insects. Some seeds may require 12 months storage before they are ripe for germination.

Step 2. Ensure the seed is viable (not dead). There are two effective means by which this may be done.

(i) An initial test for viability is to cut open 3 replicates of 30-50 seed to inspect the endosperm and embryo. If a large proportion of the batch does not have a full endosperm of healthy appearance (white colour, not floury) the batch may be of little use. One should also look for insect damage.

(ii) To determine if the seed is capable of germination it is necessary to extract the embryos of a subsample of seed and grow them *in vitro* with growth hormones (GA₃ and zeatin) using the method described in section 3.2.1. This will ensure that germination difficulties experienced with whole seed is due to dormancy issues rather than a lack of a viable embryo.

Step 3. Germination test. If a large proportion of the seed is whole with viable embryos yet germination is still low, the next step is to try smoke stimulation using the aerosol smoke tent and aqueous smoke *in vitro* (as described in section 3.2.1). It is important to determine if the seed responds to aqueous smoke as effectively as aerosol smoke. A range of dosages of aqueous smoke should be trialed to maximize germination (10-20% for 3, 6, 12 hours).

Step 4. If smoke is unable to improve germination it may be necessary to determine the site of the dormancy. This involves an *in vitro* study as described in section 3.2.2. A variety of treatments, including nicking the seed coat, removing the coat, and extracting the embryo are employed.

Step 5. Other seed pretreatments may need to be carried out in combination with smoke to break dormancy in species unresponsive to smoke alone. Seed treatments can include:

- ï Storing the seed in the soil for a year, however this often results in a simultaneous viability decline;
- ï Removal of the fruit may overcome dormancy in fleshy-fruited species as inhibitors are sometimes contained in the fruit (e.g. Epacrids such as *Astroloma foliosum*, Chapter 2; *Nitraria billardieri*, Noble and Whalley 1978);
- ï If the seed coat is a barrier to the diffusion of inhibitors from the seed or to the uptake of water, nicking the coat can alleviate this (e.g. *Emmenanthe penduliflora*, Egerton-Warburton 1997 unpubl.);
- ï If an inhibitor is contained within the seed coat the removal of the seed coat may be necessary (e.g. *Stylidium affine*, Chapter 3);
- ï Acid treatment of seeds helps to degrade the seed coat, removing inhibitors and making the coat more permeable to water (e.g. *Scaevola crassifolia*, Chapter 2).

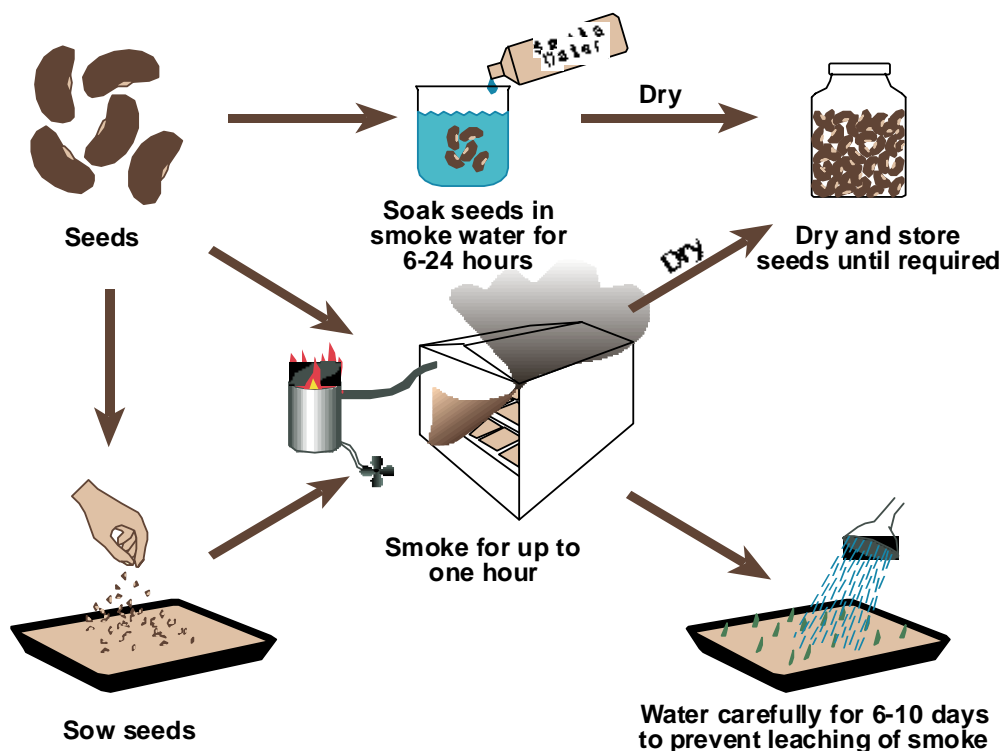


Fig. 7.16 Various methods for smoke application.

Nursery production

Once a species has been found to respond to smoke (or smoke with another germination cue) it can then be developed for nursery production or field sowing.

Aerosol smoke is the only effective treatment for some species. Other species respond equally well to aqueous smoke. These species are more adaptable for nursery production as they can be either treated directly with aqueous smoke and sown or treated and then dried for later use.

For direct use the seed should generally be imbibed in 10-20% full strength aqueous smoke for 3-6 hours or overnight. A small trial may be required to determine the optimum treatment if good results are not achieved. The seed should then be sown in punnets and germinated.

To prime seed prior to storage the seed should be imbibed in 10-20% full strength aqueous smoke but for a longer duration (12-48 hours). Following imbibition the seed should then be air dried for storage. The seed must be completely dried for storage. This can be ensured by weighing 3 replicates of seed prior to imbibition. The seed lot is dry enough when these samples dry to their original weight following imbibition. The seed can then be stored in an airtight container for at least 3 weeks.

Growing conditions

Treated seeds should be sown in punnets of potting mix to a depth equivalent to the width of the seed. Kings Park and Botanic Garden uses a mix composed of coarse sand, brown river sand and composted sawdust in a ratio of 1:3:6 with the addition of osmocote, ferrous sulphate, dolomite lime and lime. The trays should be germinated at about 18°C±1 with day/night fluctuations and regular watering.

Smoke treatment for broadacre use

Seed can be treated for direct sowing by either pre-imbibing with aqueous smoke as described above or the seed can be aerosol smoked to coat the seed with a smoke residue. The seed can be directly sown or broadcast in the field. Pre-imbibed seed is probably better if the seed is to

be mechanically sown as the sticky residue on aerosol smoked seed may cause the seed to bind together.

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