The Role of Smoke as a Germination Cue

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Photo: S. Cruickshank

You say you don't believe?
What do you call it when you
sow a tiny seed and are
convinced that a plant will grow?

~ Elizabeth York ~

Contents

Abstract

The intriguing role of smoke in stimulating seed germination was first highlighted by a study on *Audouinia capitata*, a threatened fynbos species. Further studies on South African fynbos, Californian chaparral and Australian species have illustrated the widespread ability of smoke to promote germination of many species from fire-prone areas. Interestingly, a variety of species from fire-free habitats also respond positively to smoke treatments. Consequently, aerosol smoke and smoke solutions can potentially be used for a variety of applications related to seed technology. These include uses in horticulture, agriculture, ecological management, habitat restoration and conservation.

Various investigations were conducted, on different aspects relating to smoke-stimulated seed germination, in an attempt to gain a better understanding of the action of smoke as a germination cue, and to isolate and characterise the compound(s) responsible for the stimulatory action of smoke on germination. Importantly, Grand Rapids lettuce seeds, which have a light-requirement for germination, are able to germinate within 24 h in the dark when treated with smoke solutions. Thus, Grand Rapids lettuce seeds were used as a biological test system, and formed an integral role in the experiments conducted.

Pulse-treatments of Grand Rapids lettuce seeds with smoke solutions revealed that there is a required threshold level of the active compound(s) in the seed. The retention of the germination cue by the seeds, and the reversal of the inhibitory effects of high concentrations of smoke after rinsing, suggest a dual regulatory role for smoke. This competitive interaction, in which the germination promoter(s) cannot be leached while the inhibitory compound(s) can, may be important in post-fire environments. It may provide seeds with a mechanism to prevent germination until sufficient rainfall has leached the inhibitory compound(s) away from the seeds, thereafter allowing the stimulatory compound(s), which are active over a broad concentration range, to promote germination.

Following the suggestion that smoke-stimulated seed germination is due to nitric oxide (NO), the effects of two NO-releasing compounds, N-tert-butyl-α-phenylnitrone (PBN)

and sodium nitroprusside (SNP), on the germination of Grand Rapids lettuce seeds were examined. Neither PBN nor SNP stimulated seed germination in the dark. Additionally, the NO-specific scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potassium (c-PTIO) was unable to reduce germination in response to a smoke solution. These results suggested that NO is unlikely to be responsible for the enhanced germination of Grand Rapids lettuce seeds by smoke solutions.

A highly active butenolide compound, 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one ($C_8H_6O_3$), that stimulates germination of Grand Rapids lettuce seeds in the dark, was isolated from plant-derived smoke water using bioactivity-guided fractionation. Purification steps included liquid-liquid partitioning, vacuum liquid chromatography, and HPLC fractionation. The active fractions from the final HPLC step were analysed using NMR and GC-MS. It was found that the compound promoted the germination of Grand Rapids lettuce seed over a wide range of concentrations, and at a concentration as low as 10^{-9} M.

Further experiments showed that 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one could also be formed during Maillard reactions between sugars and amino acids. Heating proteins or amino acids with sugars at 180 °C for 30 min produced water-soluble extracts that promoted the germination of Grand Rapids lettuce seeds in the dark. Using HPLC fractionation, it was demonstrated that the active compound(s) formed during these reactions co-eluted with the active fraction from a smoke extract. Analysis using GC-MS showed that the active constituent is identical to the germination cue from plant-derived smoke. The major germination cue found in smoke can therefore be formed from ubiquitously occurring organic compounds.

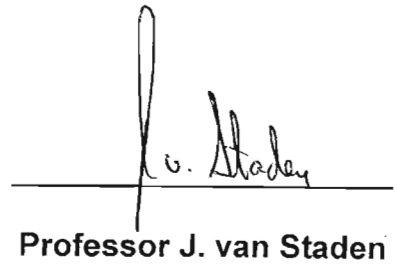
The studies presented in this thesis collectively answer several questions related to research on smoke-stimulated seed germination. The most important breakthrough is the identification of the principal germination cue from smoke, which should now lead to a more comprehensive understanding of the role of smoke as a promoter of seed germination.

Declaration

I hereby declare that this thesis, unless otherwise acknowledged to the contrary in the text, is the result of my own investigation, under the supervision of Professor J. van Staden, in the Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg:

Marnie Elizabeth Light

I certify that the above statement is correct:



Professor J. van Staden
Supervisor

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Soli Deo Gloria

Publications from this Thesis

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LIGHT, M.E. and VAN STADEN, J. (2003). The nitric oxide specific scavenger carboxy-PTIO does not inhibit smoke stimulated germination of Grand Rapids lettuce seeds. *South African Journal of Botany* 69:217-219.

LIGHT, M.E. and VAN STADEN, J. (2004). The potential of smoke in seed technology. *South African Journal of Botany* 70:97-101.

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Patents from this Thesis

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VAN STADEN, J., JÄGER, A.K., LIGHT, M.E. and BURGER, B.V. (2005). A potent germination cue from plant-derived smoke. Thirty-first Annual Conference of the South African Association of Botanists (SAAB). University of the Free State, Bloemfontein.

VAN STADEN, J. and LIGHT, M.E. (2004). Smoke-stimulated seed germination: Potential for seed technology. Seed Ecology 2004, Rhodes, Greece.

Related Publications

VERSCHAEVE, L., MAES, J., LIGHT, M.E. and VAN STADEN, J. (2006). Genetic toxicity testing of 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one, an important biologically active compound from plant-derived smoke. *Mutation Research – Genetic Toxicology and Environmental Mutagenesis* 611:89-95.

KULKARNI, M.G., SPARG, S.G., LIGHT, M.E. and VAN STADEN, J. (2006). Stimulation of rice (*Oryza sativa* L.) seedling vigour by smoke-water and butenolide (3-methyl-2*H*-furo[2,3-*c*]pyran-2-one). *Journal of Agronomy and Crop Science* 192:395-398.

KĘPCZYŃSKI, J., BIAŁECKA, B., LIGHT, M.E. and VAN STADEN, J. (2006). Regulation of *Avena fatua* seed germination by smoke solutions, gibberellin A_3 and ethylene. *Plant Growth Regulation* 49:9-16.

VAN STADEN, J., SPARG, S.G., KULKARNI, M.G. and LIGHT, M.E. (2006). Post-germination effects of the smoke-derived compound 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one, and its potential as a preconditioning agent. *Field Crops Research* 98:98-105.

SPARG, S.G., KULKARNI, M., LIGHT, M.E. and VAN STADEN, J. (2005). Improving seedling vigour of indigenous medicinal plants with smoke. *Bioresource Technology* 96:1323-1330.

GARDNER, M.J., DALLING, K.J., LIGHT, M.E., JÄGER, A.K. and VAN STADEN, J. (2001). Does smoke substitute for red light in the germination of light-sensitive lettuce seeds by affecting gibberellin metabolism? *South African Journal of Botany* 67:636-640.

JÄGER, A.K., LIGHT, M.E. and VAN STADEN, J. (1996). Effects of source of plant material and temperature on the production of smoke extracts that promote germination of light-sensitive lettuce seeds. *Environmental and Experimental Botany* 36:421-429.

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AA	atomic absorption
ABA	abscisic acid
ACC	1-aminocyclopropane-1-carboxylic acid
ANOVA	analysis of variance
BA	N^6-benzyladenine; 6-benzylaminopurine
CCC	chlorocholine chloride
CK	cytokinin
c-PTIO	2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potassium
EIMS	electron impact mass spectrometry
ESI-MS	electrospray ionisation - mass spectrometry
FR	far-red light (730 nm)
GA	gibberellin
GA_3	gibberellic acid; gibberellin A_3
$GA_{4/7}$	GA_4 and GA_7 gibberellin mixture
GC	gas chromatography
GC-MS	gas chromatography - mass spectrometry
gHMBC	gradient heteronuclear multiple bond coherence
gHSQC	gradient heteronuclear single quantum coherence
HPLC	high-performance liquid chromatography
IAA	indole-3-acetic acid
IBA	indole-3-butyric acid
IR	infrared
LY-CH	lucifer yellow carbohydrazide
NMR	nuclear magnetic resonance
NO	nitric oxide
NOESY	nuclear Overhauser effect spectroscopy
PAH	polycyclic aromatic hydrocarbon
PBN	N-tert-butyl-α-phenylnitrone
Pfr	far-red light absorbing phytochrome
PNAH	polynuclear aromatic hydrocarbon
ppm	parts per million
Pr	red light absorbing phytochrome

R	red light (660 nm)
SD	standard deviation
SE	standard error of the mean
SM	smoke solution
SNAP	*S*-nitroso-*N*-acetylpenicillamine
SNP	sodium nitoprusside
TIC	total ion chromatogram
TLC	thin layer chromatography
VLC	vacuum liquid chromatography

1 Introduction

The most exciting phrase to hear in science, the one that heralds new discoveries, is not "Eureka!" (I've found it!), but "That's funny..."

~ Isaac Asimov (1920 - 1992) ~

1.1 SMOKE AS A GERMINATION CUE

Since the landmark study of DE LANGE and BOUCHER (1990), smoke-stimulated seed germination has received increasing attention. It is a fascinating topic which encompasses various aspects of ecology and physiology and holds great potential for a variety of applications. Literature on the role of smoke as a germination cue has been reviewed (BROWN and VAN STADEN, 1997; VAN STADEN, BROWN, JÄGER and JOHNSON, 2000), and received special attention in an editorial "The Hot and the Classic" in *Plant Physiology* (MINORSKY, 2002).

1.2 AIMS AND OBJECTIVES

The overall aim of this study was to provide some answers to the intriguing nature and action of smoke as a germination cue. This was achieved through a variety of investigations on different aspects relating to this topic. Thus, the specific objectives of this study were the following:

- to investigate pulse treatments and storage treatments of Grand Rapids lettuce seeds with smoke solutions;
- to evaluate the possible role of nitric oxide in smoke-stimulated seed germination of Grand Rapids lettuce seeds;
- to isolate and identify the principal germination promoter from plant-derived smoke; and
- to investigate the role of reactions between amino acids and carbohydrates in the formation of the germination cue found in smoke.

1.3 GENERAL OVERVIEW

BASKIN and BASKIN (1998) make the comment that "although compounds that stimulate seed germination may be isolated from smoke, charred wood, or heated soil in the laboratory, their role in germination ecology cannot be understood until they have been tested in the field, which is not an easy thing to do". This is certainly true, in that there are possibly many other factors which influence germination *in situ*. However, laboratory experiments on seed germination can often lend insight into related environmental situations.

The research presented in this thesis brings together a variety of different laboratory-based studies relating to the topic of "the role of smoke as a germination cue". It represents a small portion of the work which has been conducted by our research group, at the Research Centre for Plant Growth and Development, over the last 10-12 years. Although in some respects the experiments may seem unrelated, together they begin to provide some insight towards a greater understanding of how smoke acts to promote the germination of the seeds of many species.

Chapter 2 provides a comprehensive literature review on the topic of smoke as a germination cue. As a short introduction, the role of fire in seed germination is discussed. Fire ecology is, however, an extensive topic with an enormous amount of available literature. The chapter concludes with a short discussion on the potential uses of smoke in seed technology (LIGHT and VAN STADEN, 2004).

Chapter 3 describes the "lettuce seed bioassay", used in most of the experiments, and discusses certain aspects related to the germination physiology of Grand Rapids lettuce seeds. The bioassay, developed by DREWES, SMITH and VAN STADEN (1995), has formed an integral part of the research conducted by our research group.

Chapter 4 presents results from pulse-treatments and storage-treatments of Grand Rapids lettuce seeds. It gives some evidence for the dual regulatory role of smoke in seed germination (LIGHT, GARDNER, JÄGER and VAN STADEN, 2002).

Chapter 5 presents results from an experiment which was conducted prior to the isolation of the major germination cue. The results indicated that nitric oxide was not the main active component responsible for smoke-stimulated seed germination (LIGHT and VAN STADEN, 2003).

Chapter 6 describes the isolation and identification of the principal germination cue from plant-derived smoke water. This was made possible through a collaborative effort, and is the culmination of research spanning many years (VAN STADEN, JÄGER, LIGHT and BURGER, 2004).

Chapter 7 presents results from experiments involving heating amino acids with sugars. It gives evidence that the isolated germination cue can be formed from ubiquitously occurring organic compounds (LIGHT, BURGER and VAN STADEN, 2005).

Following **Chapter 8** (General Conclusions) and **Chapter 9** (References) are two appendices, compiled from the surveyed literature. **Appendix 1** provides a list of species which responded positively to smoke treatments. In contrast, **Appendix 2** provides a list of species which *did not* show a positive response to any smoke treatments.

2 Literature Review

There can no great smoke arise, but there must be some fire.

~ John Lyly (1554 - 1606) ~

2.1 THE ROLE OF FIRE IN SEED GERMINATION

Fire is a major environmental factor, of natural or anthropogenic origin, that influences plant communities in many regions of the world. It directly affects plant growth, survival and reproduction, and also influences seeds and seedling dynamics (BOND and VAN WILGEN, 1996; KEELEY and FOTHERINGHAM, 2000). As such, fire ecology is an extensive topic which encompasses complex interactions between the major products of fire (heat, smoke and nutrients) and the environment (soil, seeds and vegetation) (DIXON and BARRETT, 2003).

Frequent and/or predictable fires occur in tropical savannas, temperate grasslands, mediterranean ecosystems and boreal (coniferous) forests - four of the world's major biomes (ARCHIBOLD, 1995). Consequently, fires form an integral part of the ecology of these ecosystems and are of particular importance in mediterranean climate regions (DI CASTRI, 1981; TRABAUD, 1981).

2.1.1 Fire in mediterranean-type regions

Mediterranean climate areas lie between 30° and 45° of latitude, near the coast along the western edges of the world's continents. This **distinctive climate pattern** is characterised by mild, rainy winters and hot, dry summers. In the northern hemisphere it is found in the region around the Mediterranean Sea and California, and in the southern hemisphere in Central Chile, the Western Cape Province of South Africa and Western and Southern Australia (DALLMAN, 1998). In these regions, the dry heat towards the end of summer and in the early autumn results in conditions which are highly susceptible to wildfires. In general, the risk of wildfires in these regions is much

greater than in adjoining semi-arid and rainforest regions, since plants in the semi-arid areas accumulate less fuel, and the rainforest is mostly too moist for wildfires (ARCHIBOLD, 1995; DALLMAN, 1998).

The major mediterranean climate regions and their **major plant communities** are summarised in **Table 2.1**. Plant communities in these regions are typically fire-adapted and have similarities in their structure and growth pattern. There are, however, also important differences in relation to the nutrient richness of the soil, the frequency of wildfires, and the prominence of annuals and geophytes (DALLMAN, 1998). The South African fynbos and the Australian kwongan have some similarities which can be attributed to the nutrient-poor soils, and neither of these plant communities is as dense as chaparral or maquis (DALLMAN, 1998). In the Chilean matorral, in contrast to other mediterranean regions, fires of natural origin are relatively infrequent because the scrub vegetation is not as dense and lightening is rare (MUÑOZ and FUENTES, 1989). Interestingly, fire-stimulated seed germination has only been shown in a few species from this region (SEGURA, HOLMGREN, ANABALÓN, FUENTES, 1998).

Table 2.1: Major forms of vegetation in mediterranean climate regions. (From DALLMAN, 1998).

Region	Shrubland	Coastal scrub	Woodland	Forest
Mediterranean	maquis	garrique	oak	oak, pine
California	chaparral	coastal sage scrub	oak	redwood
Chile	matorral	coastal matorral	sclerophyll	*Nothofagus*
Western Cape	fynbos	standveld	(scarce)	(scarce)
Australia	kwongan/mallee	kwongan	eucalyptus	eucalyptus

2.1.2 Post-fire regeneration

Post-fire regeneration is dependent on vegetative sprouting and soil- and canopy-stored seed banks (PIERCE and COWLING, 1991; DE LANGE and BOUCHER, 1993b). Patterns of persistence fall into two broad categories and are generally referred to as either resprouters or reseeders, according to which of these strategies they utilize to survive a fire (sometimes referred to as sprouters or seeders) (DALLMAN, 1998).

Following a fire, **sprouting** may occur from aerial plant parts which are undamaged by the fire, or from below ground where the soil provides some insulation (BOND and VAN WILGEN, 1996). Most plants which resprout after a fire, do so from perennial subterranean burls or root crowns (also called lignotubers), which are swollen, woody structures at the base of the trunk (DALLMAN, 1998). In Australia, the most prominent sprouters are *Eucalyptus* species. The grass tree (*Xanthorrhoea australis*) resprouts from a caudex (a reduced underground stem) that is buried about 35 cm below the soil surface (DALLMAN, 1998). Some examples of South African fynbos species that resprout following fires are *Protea cynaroides* and *Protea nitida*, and several species of *Leucadendron*. The sprouters also produce seed, but in these plants survival is not dependent on seed germination alone (HICKEY and VAN JAARSVELD, 1995).

Non-sprouting species that are killed by fire are dependent on the remaining seeds for the re-establishment of the species (HICKEY and VAN JAARSVELD, 1995). Seeds that can survive in the soil for very long periods are referred to as "refractory" or disturbance-dependent (DALLMAN, 1998). These strongly dormant seeds do not germinate unless they are stimulated by heat or chemicals released during a fire. Consequently, for many species found in these regions, fire has been shown to be a vital factor in promoting regeneration, and the majority of species rely on recruitment from soil seed banks (HOLMES and NEWTON, 2004).

The appearance of thousands of seedlings have frequently been observed following fires in mediterranean climate areas (BASKIN and BASKIN, 1998). For example, in South African fynbos, increased germination following wildfires has been observed in a number of species, including *Leucospermum cordifolium*, *Orothamnus zeyheri* and *Staavia dodii* (LEVYNS, 1935; MARTIN, 1966; SCHELPE, 1970; BOUCHER, 1981; MOLL and GUBB, 1981; BOND, VLOK and VIVIERS, 1984; BRITS, 1986). Furthermore, increased germination after fires occurs in plants of different life-history traits and growth forms, such as annuals, perennials, shrubs and trees (HORTON and KRAEBEL, 1955; SPECHT, RAYSON and JACKMAN, 1958; NAVEH, 1975; TRABAUD, 1981; MORENO and OECHEL, 1991; WILLIAMS, CONGDON, GRICE and CLARKE, 2003a).

One mode of survival following fires includes the storage of seeds in cones, woody capsules or persistent woody inflorescences that remain on the plant for many years. This adaptive strategy is termed "**serotiny**" (sometimes referred to as "bradyspory") and is found in a number of species in different mediterranean regions (LE HOUÉROU, 1981; PARSONS, 1981; BOND, 1985; LAMONT, LE MAITRE, COWLING and ENRIGHT, 1991; DALLMAN, 1998). Heat from fires opens these woody seed-storage structures, which protect and insulate the seeds, and the seeds are released to germinate on the fire-denuded and nutrient-enriched soil (BOND, 1985; BOND and VAN WILGEN, 1996; BASKIN and BASKIN, 1998; DALLMAN, 1998). A comprehensive review on canopy seed storage in woody plants has been published by LAMONT, LE MAITRE, COWLING and ENRIGHT (1991), and serotiny in the Proteaceae is also reviewed in LAMONT and GROOM (1998).

Along with species persistence, fire also influences plant **species recruitment** and the plant community structure. For example, HANES and JONES (1967) observed that chaparral vegetation in a southern California study site was altered in plant number within species and in species composition after a wildfire. In areas which are fire-prone, species are generally divided into two categories to describe their dependence on fire for recruitment. The first category includes species whose recruitment is stimulated by fire (fire-recruiters), and the second includes species which can recruit at other times in response to other cues (non-fire-recruiters). A number of species have a near obligate dependence on fire for recruitment, for example *Orothamnus zeyheri*, *Serruria florida* (BOUCHER, 1981; BOND and VAN WILGEN, 1996) and *Staavia dodii* (MOLL and GUBB, 1981). In the case of *S. dodii*, regeneration from seed is dependent on fires, although the occurrence of seedlings also appears to be related to soil moisture rather than season of burn (MOLL and GUBB, 1981).

2.1.3 Effects of fire on germination

Seed dormancy is broken by a variety of signals associated with fire. The most common factors include the **direct effects** of heat shock and/or the release of chemicals from smoke or charred wood (BOND and VAN WILGEN, 1996; KEELEY and

FOTHERINGHAM, 2000). Furthermore, the **indirect effects** associated with changes in the environment also have a large influence. These changes in the conditions of microsites include: elevated soil temperatures; increased light; changes in nitrogen content; and alteration of soil microorganisms. In addition, the combination of open space, increased resource availability and a temporary reduction in seed predators results in the post-fire environment being highly favourable for seedling establishment (BOND and VAN WILGEN, 1996; KEELEY and FOTHERINGHAM, 2000). The effects of fire on germination can be divided into various physical and chemical factors, which are diagrammatically represented in **Figure 2.1**, and discussed further.

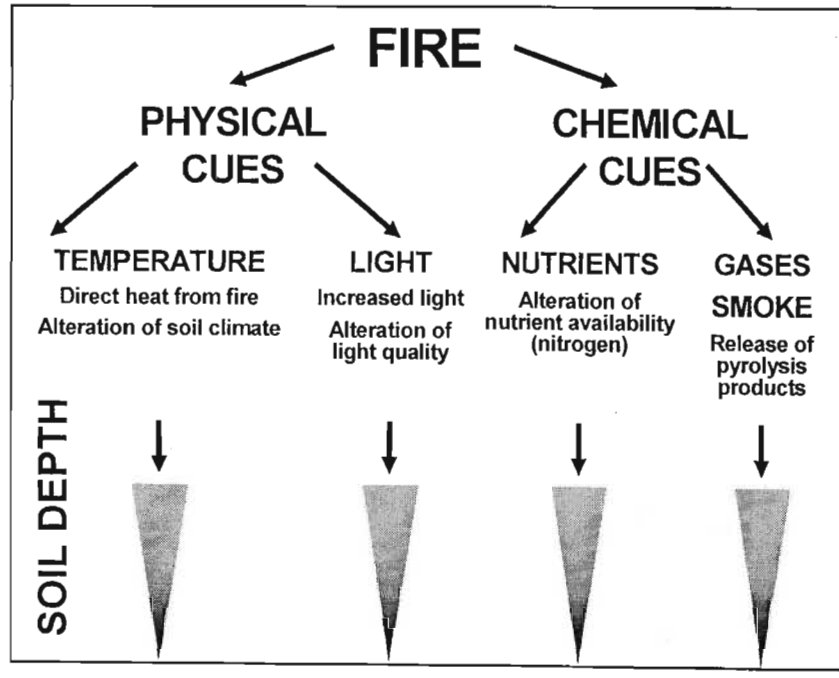

Figure 2.1: Schematic diagram illustrating the physical and chemical cues from fires influencing the seed environment. In general, the factors have the greatest influence at the soil surface, decreasing with depth. (Modified from VAN STADEN, BROWN, JÄGER and JOHNSON, 2000).

2.1.3.1 Physical effects of fire on germination

The **direct effect of heat** permeating the soil during a fire has been implicated in the germination response of many species, particularly in legumes and other hard-seeded species (DIXON and BARRETT, 2003). Soil temperatures during fires depend on the characteristics of the fire, which are related to the fuel type and load, and vary according

to soil depth (BRADSTOCK, AULD, ELLIS and COHN, 1992; DE LANGE and BOUCHER, 1993b; ODION and DAVIS, 2000). Soil temperatures decrease with soil depth, although the duration of elevated temperature increases with soil depth. Intense fires in mediterranean-type vegetation can result in soil surface temperatures that may reach well over 700 °C with arrival of the flame front, but which only last for 10-15 s. After 3-5 min, the temperature drops to 500-600 °C, although this degree of heat is not sustained for a long time as the fire rapidly consumes the surface fuel and moves on (MARTIN, 1966; ODION and DAVIS, 2000). Below the surface, at depths of 2 cm the temperatures are considerably lower and may be between 50 and 120 °C (MARTIN, 1966; BRADSTOCK, AULD, ELLIS and COHN, 1992; DE LANGE and BOUCHER, 1993b).

Numerous studies have been conducted on the effects of **heat-shock** on seed germination. For example, heat-stimulated germination has been demonstrated in members of the Asteraceae, Cistaceae, Convolvulaceae, Ericaceae, Fabaceae, Malvaceae, Proteaceae, Restionaceae, Rhamnaceae and Sterculiaceae (BLOMMAERT, 1972; VAN DE VENTER and ESTERHUIZEN, 1988; MUSIL, 1991; MUSIL and DE WITT, 1991; KEELEY and FOTHERINGHAM, 2000). BASKIN and BASKIN (1998) provide an extensive list of species on which the effects of dry heat treatments on germination have been tested. The list includes those species which have shown an increase in germination, those which resulted in decreased germination, and species in which the germination was not affected. Many species which exhibit increased germination following exposure to temperatures between 80 and 120 °C belong to families possessing physical dormancy (BASKIN and BASKIN, 1998). These high temperatures may cause the seed or fruit coats to become water permeable (i.e. heat-induced scarification), thereby allowing subsequent imbibition and germination (MARGARIS, 1981). There are some species, however, which possess physical dormancy mechanisms that do not necessarily respond to heat treatments. Furthermore, there are some species with physiological dormancy that do respond to heat treatments (BASKIN and BASKIN, 1998).

The removal of litter, shrubs and canopies due to a fire, results in an **increased amount of light** (and heat) reaching the soil surface. The magnitude of this increase, however,

depends on the vegetation type and the characteristics of the fire. This has a two-fold effect of changing the soil climate and exposing seeds near the soil surface to increased irradiance. KEELEY (1984) showed that light is a significant factor in the germination of seeds of chaparral species found in soils sampled from unburned and burned sites. TYLER (1995), however, observed no increases in germination of seeds of annuals, perennials and subshrubs by the removal of shrubs in field studies in California chaparral. Interestingly, in a study on the effects of light quality on seed germination of species from Western Australia, it was observed that the importance of light depended on the size of the seed, its life form and the species habitat (BELL, KING and PLUMMER, 1999).

The loss of green vegetation also **alters the light quality** by changing the red:far-red light ratio at the soil surface. ROY and ARIANOUTSOU-FARAGGITAKI (1985) found that by removing green leaves from phrygana vegetation in Greece, the red:far-red ratio increased from 0.3 to 1.1 and the germination of seeds of the dwarf shrub *Sarcopoterium spinosum* increased from 3% to 30%.

The **alteration of the soil climate** due to vegetation loss is one of the most striking effects of fires. Following a fire, there is an increase in soil surface temperature and an increase in daily temperature fluctuations. AULD and BRADSTOCK (1996) measured the patterns of soil temperatures to a depth of 5 cm, after a summer fire, a winter fire, and in an unburnt sclerophyll woodland during summer. Following a summer fire, maximum temperatures above 60 °C were only found in the top 0.5 cm of soil, and temperatures above 40 °C were found up to 4.5 cm in depth. In comparison, the surface temperature of the unburned site was less than 40 °C, decreasing to approximately 30 °C at a depth of 1.0 cm (AULD and BRADSTOCK, 1996). Similarly, BRITS (1986) observed greater temperature fluctuations in a burned area of mesic mountain fynbos, the conditions of which meet the germination requirements for *Leucospermum cordifolium* and *Serruria florida* seeds.

2.1.3.2 Chemical effects of fire on germination

During a fire many **volatile compounds** are produced from the combustion of organic matter, which may directly affect soil and canopy-stored seeds. For example, indirect evidence was obtained for **ethylene** and **ammonia** as possible volatile cues stimulating the germination of *Erica hebecalyx* seeds (VAN DE VENTER and ESTERHUIZEN, 1988). These factors, however, have received less attention in research related to fire ecology than heat-shock effects. The release of a compound in **smoke**, which is now known to play a major role as an important germination cue, is discussed further in subsequent sections.

Charred wood, or leachates from charred wood, also appear to be important in promoting germination following a fire. BASKIN and BASKIN (1998) provide a comprehensive summary of examples where charate or powdered charred wood has been shown to stimulate seed germination in matorral species. In some species, however, charate had no stimulatory effect on seed germination, and in other cases, germination was decreased (also see **Appendix 1** and **Appendix 2**).

WICKLOW (1977) observed that seeds of the chaparral herb *Emmenanthe penduliflora* germinated within four weeks when placed immediately adjacent to burned stem segments of the chaparral shrub *Adenostoma fasiculatum*. In contrast, completely ashed remains of these stems, or unburned stems, did not promote germination (WICKLOW, 1977; JONES and SCHLESINGER, 1980). Field studies also showed that *E. penduliflora* germinated in sites on which the brush was burned in windrows, as well as in sites on which the cold remains of burned brush had been deposited. Thus, WICKLOW (1977) concluded that the charred remains of chaparral vegetation provided a trigger for germination of seeds of this shrub.

KEELEY and NITZBERG (1984) further examined the charred wood enhancement of seed germination in *E. penduliflora* and *Eriophyllum confertiflorum*. In the presence of charate (powdered charred stems of *A. fasciculatum*), germination of *E. penduliflora* and *E. confertiflorum* was significantly improved. An increase in germination, however, was

not observed with treatments using activated charcoal. Importantly, KEELEY and NITZBERG (1984) demonstrated that a water extract of the charate enhanced germination as well as or better than the directly applied charate. Also of interest was the increased germination obtained with heated chaparral soil (195 °C for 10 min), or sterile extracts thereof. This germination activity was most likely due to organic matter in the soil being heated.

In a study on other chaparral herbs and suffrutescents, KEELEY, MORTON, PEDROSA and TROTTER (1985) showed that the germination of some species was markedly stimulated by heat, charred wood, or both. KEELEY and KEELEY (1987) tested 57 chaparral herbs and suffrutescents for their response to charred wood and heat shock. Of the species tested, five herbaceous perennials and suffrutescent species and 20 of the annual species were significantly stimulated by charred wood. In another study on 45 species of woody taxa from coastal sage scrub and chaparral, KEELEY (1987) showed that charate significantly enhanced the germination of approximately 25% of the species tested. In several species, charate was able to overcome dark inhibition. Similarly, KEELEY (1986) also showed that seeds of *Salvia mellifera*, which failed to germinate in the dark, were able to do so when exposed to charred wood.

Although the majority of studies related to charate-stimulated germination have focussed on chaparral species, the effect is not limited to species from this region. For example, BROWN (1993b) showed that extracts of charred wood significantly improved germination in *Syncarpha vestita* to equivalent levels obtained with treatments using smoke solutions. In a study on the effects of seed storage and fire on the germination in a nut-fruited Restionaceae species, *Cannomois virgata*, it was shown that both fire- and charate-treated seeds exhibited significantly higher germination than the control (NEWTON, BOND and FARRANT, 2006).

Charate treatments also improved the germination of some Mediterranean species (PÉREZ-FERNÁNDEZ and RODRÍGUEZ-ECHEVERRÍA, 2003). However, it is also important to note that charred wood failed to enhance the germination of 40 non-leguminous understorey species of the northern jarrah forest of Western Australia, with the exception of *Burchardia umbellata* (BELL, VLAHOS and WATSON, 1987). Similarly,

of 22 phrygana species from the eastern Mediterranean Basin, only *Lavendula stoechas* showed a response to charred wood treatment (KEELEY and BAER-KEELEY, 1999).

KEELEY and PIZZORNO (1986) demonstrated that charred wood produced from a variety of chaparral and non-chaparral woods were equally effective at promoting germination of *E. penduliflora* and *E. confertiflorum* seeds. Furthermore, KEELEY and PIZZORNO (1986) found that wood heated to 175 °C for 30 min produced the same germination stimulation. From these studies (eg. KEELEY and NITZBERG, 1984; KEELEY and PIZZORNO, 1986) it appears that the improved germination is attributable to a water soluble compound(s) that is leached from the charred wood. Furthermore, these studies suggest that the compound can be produced by heating or charring any type of wood (also see **Section 7.1.2**). However, if the wood is combusted to ash, the compound is destroyed or volatilised. Given the current knowledge on the smoke-derived germination cue, it is most likely that the stimulatory compound in charred or heated plant material is the same as that found in smoke (also see **Chapter 6** and **Chapter 7**; FLEMATTI, GHISALBERTI, DIXON and TRENGOVE, 2004; VAN STADEN, JÄGER, LIGHT and BURGER, 2004; LIGHT, BURGER and VAN STADEN, 2005).

Fires also result in a number of **chemical changes in the soil**. This can be attributed to three processes: (1) the combustion of organic matter in the soil; (2) the input of ashes; and (3) the alteration of nutrient availability (DIAZ-FIERROS, BENITO, VEGA, CASTELAO, SOTO, PÉREZ and TABOADA, 1990). The literature on post-fire soil nutrient availability is substantial and is important for understanding the role of fire in nutrient recycling processes and the role of ashbeds in promoting seedling growth (DIXON and BARRETT, 2003).

The development of seedlings, following a fire, largely depends on the response of seeds to conditions in the upper layer of the soil. **Ash**, which can sometimes form a thick layer above the soil, strongly affects the **pH** and **osmotic potential** of the soil water (HENIG-SEVER, ESHEL and NE'EMAN, 1996). Furthermore, ash contains high levels of nitrogen, thereby providing a reservoir of organic nitrogen (CHRISTENSEN, 1973). Several studies, as discussed in REYES and CASAL (1998), have demonstrated

a positive effect of ash on the regeneration of different species following fires. Improved regeneration of some species may be attributable to the increase in pH and release of nutrients into the soil from burned shrubs and leaf litter (DALLMAN, 1998; REYES and CASAL, 1998). However, high pH may inhibit radical elongation of germinating seeds, thereby reducing germination (HENIG-SEVER, ESHEL and NE'EMAN, 1996).

In contrast, other studies have indicated that ash does not appear to play a direct role in promoting germination. GONZÁLEZ-RABANAL and CASAL (1995), in a study on the effect of high temperatures and ash on the germination of 10 gorse shrubland species, found that treating seeds with an aqueous suspension of mixed ash reduced the germination. Similarly, no improvement in the germination of *Themeda triandra* (BAXTER, VAN STADEN, GRANGER and BROWN, 1994) or *Nicotiana attenuata* (BALDWIN, STASZAK-KOZINSKI and DAVIDSON, 1994) was observed with ash treatments. REYES and CASAL (1998) also did not observe any positive effect of ash on the germination of seeds of three forest species.

Another important effect of fire relates to changes in the **available nitrogen** content of soils. As mentioned above, nitrogenous compounds are released from the combustion of living and dead plant material, and their availability is increased in the soil with the addition of ash. Some of the nitrogen is volatilized and lost from the site (DEBANO and CONRAD, 1978), but in general, soil nitrogen content increases immediately after a fire as nitrogen is mineralized and added to the soil in the form of ammonium (CHRISTENSEN and MULLER, 1975; STOCK and LEWIS, 1986; LYNDS and BALDWIN, 1998). Over time, the ammonium is oxidized and/or biologically nitrified, thereby increasing the levels of nitrate in the soil (CHRISTENSEN, 1973; LYNDS and BALDWIN, 1998).

It is well known that **nitrogenous compounds** (nitrite and nitrates) are effective in overcoming dormancy and/or promoting germination in seeds of many species (BASKIN and BASKIN, 1998). For example, various species with light-sensitive seeds respond to different nitrates or nitrites (TOOLE, HENDRICKS, BORTHWICK, and TOOLE, 1956). The germination of several Western Australian species is also enhanced by potassium nitrate (BELL, KING and PLUMMER, 1999). The promotive effect of nitrate

is affected by various environmental factors, including light and temperature, as seen in *Capsella bursa-pastoris* (POPAY and ROBERTS, 1970), *Chenopodium album* (ROBERTS and BENJAMIN, 1979), *Avena fatua* (HILTON, 1984; SAINI, BASSI and SPENCER, 1985) and *Sinapis arvensis* (GOUDEY, SAINI and SPENCER, 1987).

In a study on chaparral species, THANOS and RUNDEL (1995) showed that the application of nitrates promoted germination of seeds of *Emmenanthe penduliflora*, *Phacelia grandiflora*, and to a lesser extent, *Salvia mellifera*. Ammonium was also effective in promoting the germination of *P. grandiflora* and *S. mellifera* seeds. Although extracts of charred wood also improve germination in these species, the concentration of nitrogenous compounds in charate extracts are too low to account for the stimulatory effect of charate. However, the concentration of nitrate and ammonium required for germination were of a similar level to that found in chaparral soil following a fire, and it was concluded that nitrogenous compounds play an important role in post-fire germination of these species (THANOS and RUNDEL, 1995). KEELEY and FOTHERINGHAM (1998b) also observed that KNO_3 promoted germination in several chaparral species. For *Silene multinervia*, germination was slightly stimulated by nitrate, although not to the level of germination induced by smoke.

Fires also have an impact on **soil microorganisms**, and the increase in soil pH after fires generally favours the growth of nitrifying and nitrogen-fixing bacteria (CHRISTENSEN, 1973). In a study on chaparral soils, it was observed that the numbers of bacteria and fungi were higher in burned than unburned soils (CHRISTENSEN and MULLER, 1975). ACEA and CARBALLAS (1996) investigated the fluctuations of soil microorganisms involved in carbon and nitrogen cycles following a fire in a *Pinus pinaster* forest in Spain. The different physiological groups of microorganisms were estimated 1 month and 1 year after the fire. Analyses, both in the field and during soil incubations, demonstrated that cellulase-producers decreased whilst amylase-producers and ammonifying microorganisms increased in the short term. In a study investigating the effects of experimental fire on soil microorganisms in tropical savannah woodland, ANDERSSON, MICHELSEN, JENSEN and KJØLLER (2004) demonstrated that microbial biomass was higher in burned than unburned plots 12 days after burning. However, addition of extra biomass (i.e. increased fuel load) led to a

reduction of microbial biomass as a result of an increase in fire temperature. This study concluded, however, that low intensity fires induced changes in microbial biomass that were relatively small in comparison to seasonal variations. It is also of consideration that some soil microorganisms produces substances that may inhibit germination (KAMINSKY, 1981; KEELEY and NITZBERG, 1984). Thus, the destruction of these allelopathic chemicals (or the microorganisms that produce them), may allow for improved germination of certain seeds (McPHERSON and MULLER, 1969).

Overall, it is generally viewed that natural fires are essential for maintaining species diversity in many ecosystems. The various direct and indirect factors associated with fires, as discussed above, result in post-fire conditions that greatly influence post-fire germination and the subsequent plant community development. All the different factors are highly variable and interactive, thus making the study of fire ecology so dynamic.

2.2 GERMINATION RESPONSES TO SMOKE

As discussed in the previous section, many factors associated with fire affect the germination of seeds of species from fire-prone regions. Research on fire ecology has largely focussed on plant responses to heat and nutrient release during and after a wildfire, and the role of other products of fire (gases and smoke) were mostly overlooked (VAN DE VENTER and ESTERHUIZEN, 1988; DIXON and BARRETT, 2003). Although germination has been reported for many species following wildfires, the effect was, in most cases, ascribed to the heat or desiccating effect of fire. VAN DE VENTER and ESTERHUIZEN (1988) were among the first to point out that high temperatures were not necessarily the only factors associated with fire that stimulated germination, and that there could be multiple germination cues associated with fire.

2.2.1 The discovery of smoke as a germination cue

In 1990, DE LANGE and BOUCHER demonstrated that smoke, in particular, was responsible for the germination stimulation of a threatened monotypic fynbos species, ***Audouinia capitata*** (Bruniaceae). It was found that cooled smoke stimulated the germination of natural populations of *A. capitata* under field conditions. This was

achieved by passing smoke, generated from burning a mixture of fresh and dry fynbos material, into a small plastic tent erected above the soil, a method which eliminated any possible heat effect. Furthermore, DE LANGE and BOUCHER (1990) also showed that smoke solutions could be prepared by bubbling smoke through water. Soaking seed-containing fruits in this aqueous smoke extract was as effective as either direct smoking of the soil, or a gibberellic acid treatment, in promoting the germination of *A. capitata*.

Following the significant discovery by DE LANGE and BOUCHER (1990), numerous other species, mainly from South African fynbos, Western Australia and Californian chaparral, have been tested for a germination response to treatments using aerosol smoke or smoke solutions. The different methods of smoke application are discussed in **Section 2.3.2**. Studies investigating smoke-stimulated seed germination have adopted a wide variety of methodological approaches, including *in situ* (in natural habitat bushland) and *ex situ* (nursery and laboratory glasshouse) experiments, and stimulation of seeds in the soil seed bank has been reported (also see **Section 2.2.7.2**; DIXON, ROCHE and PATE, 1995; ROCHE, DIXON and PATE, 1998).

From the literature surveyed, two appendices have been compiled listing species which have been tested for a germination response to smoke treatments. **Appendix 1** lists species whose seeds are stimulated by aerosol smoke or smoke solutions. It also includes species which have shown a response to charred wood or extracts thereof, due to the likelihood that the stimulatory compounds in smoke are also responsible for the stimulatory action of charred wood (DIXON, ROCHE and PATE, 1995). **Appendix 2** lists species which did not show a significant germination response to smoke treatments (or charred wood treatments).

2.2.2 Studies on South African species

Fynbos, a community of small shrubs, evergreen and herbaceous plants and bulbs, is the dominant vegetation type of the Cape Floral Kingdom, the smallest of the world's six floral kingdoms. The **Cape Floristic Region** contains an extremely rich flora, with approximately 8600 species, of which more than two-thirds are endemic to the region

(BROWN, 1999). As discussed in **Section 2.1.1**, fires are common in fynbos vegetation and it is rare to find fynbos stands that are more than 20 years old, with periodic fires occurring every 5 to 40 years (KRUGER and BIGALKE, 1984; DALLMAN, 1998).

Fynbos is characterized by the representation of a large number of species from the following families: Asteraceae, Bruniaceae, Ericaceae, Proteaceae, Rutaceae and the Restionaceae. In particular, there is enormous diversity in the genera *Protea* and *Erica*, and the rush-like plants of the Restionaceae (TAYLOR, 1978; BOND and GOLDBLATT, 1984; DALLMAN, 1998). Approximately 1400 of the species from these and other fynbos families are categorized as being rare, threatened or endangered (BROWN, BOTHA, KOTZE and JAMIESON, 1993).

BROWN (1993a) tested 28 fynbos species, from seven families, for a germination response to smoke and/or smoke solutions. Of the species tested, 12 showed a significant improvement in germination. The most striking response was observed in *Syncarpha vestita*, which showed an increase in germination from 5% to 95% (BROWN, 1993a). An extract from charred wood also improved germination of this species to a similar extent (BROWN, 1993b). Further studies on species from the Ericaceae family showed that 26 of the 40 species tested gave a significant germination response to an aerosol smoke treatment (BROWN, KOTZE and BOTHA, 1993). Amongst the species responding to the smoke treatment were some species of particular horticultural importance, including *Erica curvirostris*, *Erica formosa*, *Erica glomiflora*, *Erica oatesii*, *Erica pinea* and *Erica phylicifolia*.

It is important to note, however, that smoke did not promote seed germination in all species. For example, some *Erica* spp. (BROWN, KOTZE and BOTHA, 1993), *Calopsis impolita*, *Phylica pubescens* (BROWN, 1993a), *Caryotophora skiatophytoides*, and *Saphesia flaccida* (HICKEY and VAN JAARSVELD, 1995) did not show improved germination with smoke treatments.

The **Restionaceae** are reed-like plants that vary in height from 20 cm to over 3 m. In South Africa there are about 300 species that are endemic to the Cape Floristic Region

(GOLDBLATT, 1978). They are important horticultural plants that are sought after for their sculptural form and attractive, long-lasting seed heads (JAMIESON and BROWN, 1995). Few dormancy-breaking treatments, however, have been successful for species of Restionaceae (BROWN, JAMIESON and BOTHA, 1994). Consequently, exploitation of their horticultural potential, and conservation, has been limited.

Following initial findings by BROWN (1993a) that smoke and/or smoke solutions stimulated the germination of four out of ten Restionaceae species tested, a further 32 species were tested (BROWN, JAMIESON and BOTHA, 1994). Of these species, 25 showed a statistically significant increase in germination following smoke treatment, with 16 showing a particularly improved germination response. The four species that did not germinate were *Cannomois parviflora*, *Hypodiscus neesii*, *H. striatus* and *Willdenowia incurvata*, all myrmecochorus, nut-fruited species.

PIERCE, ESLER and COWLING (1995) investigated the effect of smoke water on members of the family **Mesembryanthemaceae**, of which there are more than 2000 species in South Africa. To compare the response of species from fire-prone and fire-free habitats, 16 fynbos (fire-prone) and six karroo (non-fire) species were selected. Approximately half the fire-prone species showed some response to smoke and all but one of the non-fire species showed a significant improvement in germination. Thus, the effect of smoke in promoting germination is not restricted to species found in fire-prone environments.

In addition to fynbos species, smoke treatments were able to overcome dormancy in the montane **fire-climax grass** *Themeda triandra* (BAXTER, VAN STADEN, GRANGER and BROWN, 1994). Seeds treated with a smoke solution showed increased germination at an optimum temperature of 30 °C (3% to 36%), and also at a suboptimal temperature of 15 °C (2% to 22%).

BROWN and BOTHA (2002) have recently published an updated list of fynbos species which have seeds that respond favourably to smoke treatments. The seeds of more than 300 fynbos species have been tested for a germination response to smoke or aqueous smoke extracts. Of these, **157 species** have shown a positive response and

include members of the Asteraceae (everlastings), Bruniaceae (brunias), Campanulaceae, Caryophyllaceae, Crassulaceae (crassulas), Ericaceae (ericas, Cape heaths), Fabaceae, Geraniaceae (pelargoniums), Haemodoraceae, Iridaceae, Mesembryanthemaceae (mesembs), Molluginaceae, Penaeaceae, Poaceae (grasses), Polygalaceae, Proteaceae (proteas, sugarbushes, pincushions and conebushes), Restionaceae (restios, Cape reeds or grasses), Rosaceae, Rutaceae, Scrophulariaceae, Stilbaceae and Thymelaeaceae. Interestingly, although a positive germination response to smoke is found in a wide range of families, not all species within a family or genus exhibit the same response (BROWN, VAN STADEN, DAWS and JOHNSON, 2003).

BROWN, VAN STADEN, DAWS and JOHNSON (2003) studied collated germination data from studies on fynbos species which have been tested for smoke-stimulated seed germination. The germination responses were examined for any patterns associated with **plant life-history traits**. In general, Amaryllidaceae and Hyacinthaceae species, and the majority of Iridaceae species tested (12 out of 14), do not respond to smoke treatments (BROWN, VAN STADEN, DAWS and JOHNSON, 2003). It was found that seeds of herbaceous perennials were more likely to respond to smoke treatments, and geophytes are less likely to respond than other species. Furthermore, plants with some capacity to resprout were less likely to respond to smoke than obligate seeders. Hence, serotinous species were less likely to respond than non-serotinous species. In agreement with DIXON and ROCHE (1995), it was also observed that seed mass was not a reliable predictor of a germination response to smoke treatments.

2.2.3 Studies on Australian species

In Australia, researchers at King's Park and Botanic Gardens have contributed significantly to investigating the effect of smoke-stimulated seed germination, particularly on species from **Western Australian** plant communities. The germination enhancement of Australian species by smoke has now been reported in more than 170 species from 37 families (ROCHE, DIXON and PATE, 1997; BELL, 1999).

DIXON, ROCHE and PATE (1995) tested 94 species, from 30 families of native

Western Australian plants that are usually difficult to germinate. Experiments using cooled aerosol smoke, showed that 45 of these species responded positively to the smoke treatments. From this group of smoke-responsive species, 23 had previously been recorded as extremely difficult to germinate using conventional methods, and include members of the Rutaceae, Dilleniaceae, Proteaceae, Myrtaceae, Cupressaceae and Thymelaeaceae. Species which showed a markedly positive response to smoke include *Anigozanthos manglesii*, *Burchardia umbellata*, *Conostylis setosa*, *Gelznowia verrucosa*, *Grevillea wilsonii*, *Lechenaultia bioloba*, *Pimelea spectabilis*, and *Thysanotus multiflorus*.

ROCHE, DIXON and PATE (1997) examined 180 species for smoke-stimulated germination in a study which also looked at the effect of seed ageing related to smoke responsiveness. Aerosol smoke treatment of fresh seeds, after sowing, significantly improved the germination in 97 species, and a further 20 showed improved germination. Of these, 26 species showed an absolute requirement for smoke (i.e. they did not germinate without smoke treatment, regardless of any other ageing treatment).

ROCHE, KOCH and DIXON (1997) investigated the use of smoke for enhancing seed germination for **mine rehabilitation** in Australia (also see **Section 2.6.6**). Application of aerosol smoke to unmined *Eucalyptus marginata* (jarrah) forest soils resulted in a 48-fold increase in total germinants and a four-fold increase in species number. Significantly higher germination was observed for 21, out of 55 species (38%). Similarly, treatment of rehabilitated mine soils also showed an increase in the total germinants and species number, although much lower in comparison to forest soils. The treatment of mixed seed lots with aerosol smoke, before broadcast, resulted in an increase of 85% of the total number of germinants and 34% increase in species richness, in comparison to the control. In the same study, smoke water was applied to the soil seed bank of previously mined sites, resulting in an increase in total germinants and species number by 56% and 33%, respectively. From this study, ROCHE, KOCH and DIXON (1997) concluded that aerosol smoking of seed before broadcast was the most effective approach for improving germination rate and species composition for bauxite mine rehabilitation. It is also interesting to note that six species, present as topsoil stored seed, showed a significant positive response to smoke residue transferred to the soil via

aerosol smoked seed of different species.

In a study of **forest topsoil**, READ, BELLAIRS, MULLIGAN and LAMB (1999) showed that members of the Poaceae were the most responsive to smoke treatments, relative to the other taxa present. READ and BELLAIRS (1999) tested seeds of 20 native **grass species** (14 genera) from New South Wales for a germination response to smoke treatments. The interaction between husk-imposed dormancy was also examined for 16 species by removing the floral structures surrounding the seeds. Almost half of the species tested showed a significant increase in germination in response to smoke treatments, with *Panicum decompositum* showing the greatest response. Interestingly, the response differed between different genera and between species of the same genus. For five species, although the final germination percentage was not significantly different, smoke treatments increased the germination rate (also see **Section 2.5.5**). Retention of the covering structures did not prevent smoke-stimulated germination for the species which responded to smoke treatments. CLARKE and FRENCH (2005) tested 22 members of the Poaceae (20 native species, two exotic) from an endangered grassy woodland community in eastern Australia for germination responses to smoke and/or heat treatments. It was found that germination of five species was significantly promoted by smoke, and for six species the effect of smoke varied with temperature.

Several studies on Australian species have investigated the effect of smoke in combination with heat treatments. **Section 2.2.8** gives more information on these studies and discusses the interactions of smoke and heat as partner cues in post-fire regeneration.

2.2.4 Studies on species from other regions

Several species from **California chaparral** have also shown a germination response to smoke treatments. KEELEY and FOTHERINGHAM (1997) found that *Emmenanthe penduliflora*, which was previously shown to be stimulated by extracts from charred wood (see **Section 2.1.3.2**), also germinated in response to smoke treatments. KEELEY and FOTHERINGHAM (1998b) tested 34 chaparral species and found that

smoke significantly increased germination in 22 species, from 11 families. KEELEY, McGINNIS and BOLLENS (2005) tested eight chaparral species from Sierra Nevada for the effect of smoke solution treatments, as well as heat-shock treatments (see **Section 2.2.8**). Of the species tested, two shrubs and two herbs exhibited significantly greater germination for one or more of the smoke treatments.

The post-fire annual ***Nicotiana attenuata***, a tobacco native to the Great Basin desert, has also been shown to have a significant germination response to smoke treatments (BALDWIN, STASZAK-KOZINSKI and DAVIDSON, 1994; BALDWIN and MORSE, 1994). Consequently, it has been used in several studies investigating the role of fire and smoke as a germination cue (LYNDS and BALDWIN, 1998; PRESTON and BALDWIN, 1999; KROCK, SCHMIDT, HERTWECK and BALDWIN, 2002; PRESTON, BECKER and BALDWIN, 2004; SCHWACHTJE and BALDWIN, 2004).

Aerosol smoke treatments have shown improved germination in several species found in shrubby woodlands in central-western **Spain** (PÉREZ-FERNÁNDEZ and RODRÍGUEZ-ECHEVERRÍA, 2003). Some species from the central **Mediterranean Basin**, also showed a positive response to aerosol smoke treatments (CROSTI, LADD, DIXON and PIOTTO, 2006). In this study, although the final germination values of some species were not significantly different, the emergence of the seedlings was more rapid in comparison to control seedlings.

RAZANAMANDRANTO, TIGABU, SAWADOGO and ODÉN (2005) have also investigated the effect of smoke treatments on germination of eight savanna-woodland species from **West Africa**. Although germination was not significantly improved in most of the species tested, almost all the smoke treatments resulted in a significantly shortened mean germination time in comparison to the controls.

2.2.5 Responses of vegetable seeds

The promotion of germination by smoke is not limited to species from fire-prone habitats, and some commercial and agricultural species also respond positively to

smoke treatments. For example, DREWES, SMITH and VAN STADEN (1995) demonstrated that light-sensitive lettuce seeds (*Lactuca sativa* cv. Grand Rapids) responded to treatments with smoke solutions. This ability of smoke solutions to substitute for the light requirement of these seeds, and their rapid germination, resulted in Grand Rapids lettuce seeds being used as a bioassay for detection of the germination cue(s) found in smoke solutions. This is discussed further in **Chapter 3**. In celery (*Apium graveolens*), another light-sensitive species, dormancy was broken in the dark with a combination of gibberellins ($GA_{4/7}$) and smoke solution or N^6-benzyladenine (BA) and smoke solution (THOMAS and VAN STADEN, 1995).

In a study on two traditional landraces of maize (*Zea mays*), seeds treated with wood fire smoke during storage had significantly higher germination than untreated seeds (MODI, 2002). Similarly, SPARG, KULKARNI and VAN STADEN (2006) demonstrated that both smoke water and aerosol smoke improved the germination of a commercial maize cultivar. Furthermore, in both these studies, seedling vigour was improved as a result of treatments with smoke (see **Section 2.5.5**).

2.2.6 Responses of weed species

It is of interest that the seeds of several weed species, many from non-fire prone regions, respond to various smoke treatments. For example, in a study on soil seed-banks from a rehabilitated mine area in Western Australia, GRANT and KOCH (1997) found that of the 13 species which showed significantly higher germination as a result of smoke treatment, six were weed species. DOHERTY and COHN (2000) investigated the effect of smoke treatments on grains of red rice (*Oryza sativa*), a problematic weed in rice production areas. The dormancy of both intact and dehulled grains was broken with smoke solutions, although the intact grains needed to be pricked to elicit this response. Consequently, smoke solutions may prove to be a useful management tool for the control of weeds (discussed further in **Section 2.6.4**).

In a study of arable and rangeland weeds, ADKINS, DAVIDSON, MATTHEW, NAVIE, WILLS, TAYLOR and BELLAIRS (2000) found that smoke solutions stimulated the

germination of two monocot weeds, namely *Avena sterilis* spp. *ludoviciana* (wild oat) and *Phalaris paradoxa* (paradoxa grass). In addition, soil seed bank studies showed a significant emergence response to smoke stimulation for three weed species (*Melinus minutiflora*, *Panicum maximum*, and *Verbena officinalis*). A further study by ADKINS and PETERS (2001) on arable weeds also demonstrated the stimulatory effect of smoke solutions on partly after-ripened caryopses of nine different biotypes of *Avena fatua* (wild oat). Smoke water treatments were also tested on 18 other weed species. In general, the dormant monocotyledonous species were strongly stimulated by smoke solutions. The dicotyledonous species, however, differed in their response to smoke water treatments. Species were either strongly stimulated (*Malva neglecta*), moderately stimulated (*Veronica persica*, *Galium aparine*), slightly stimulated (*Polygonum persicaria*, *P. pennsylvanicum*, *Fallopia convolvulus*), unaffected (*P. aviculare*, *Sinapis arvensis*, *Mercurialis annua*, *Veronica hederifolia*) or inhibited (*Lamium purpureum*).

2.2.7 General comments and considerations

2.2.7.1 Concentration effects

It is important to note that the response of seeds to smoke is affected by the **duration of exposure** to aerosol smoke, or the **concentration of the smoke solution**. For example, KEELEY and FOTHERINGHAM (1998b) found that a 15 min exposure to aerosol smoke was optimum for germination of *Romneya coulteri*, but lethal to seeds of *Emmenanthe penduliflora*. Thus, species differ in the concentration of chemicals in smoke required to stimulate germination, and also differ in their tolerance to the duration of smoke exposure (KEELEY and FOTHERINGHAM, 1998b). Consequently, understanding germination responses to smoke is sometimes complicated because species differ in the level of smoke products that are stimulatory to germination, and in the level that may be inhibitory to the seeds (also see **Section 4.1**). Thus, in order to correctly interpret negative responses, it is important that experimental determination of smoke-stimulated germination include a **dosage-response curve** (KEELEY and FOTHERINGHAM, 2000; KEELEY, McGINNIS and BOLLENS, 2005). For example, in a study on species from grassy woodlands and forests in New South Wales, CLARKE,

DAVISON and FULLOON (2000) found that several genera that have previously been reported to respond to smoke treatements did not show any pronounced germination effects. In this study, smoke treatments (1:10 dilution of smoke water) most often resulted in inhibition. Thus, in this case, it is possible that the concentration of smoke solution may have been too high, especially considering that the germination treatments were conducted in germination dishes.

ADKINS and PETERS (2001), observed **root damage** and a **reduction in germination** for caryopses of *Avena fatua* treated with high concentrations of smoke water. At optimal concentrations, however, germination was 92%. Thus, at high concentrations, the promotive effective of smoke is diminished by toxic side-effects. It was found that the toxic effects could be avoided by first imbibing the caryopses on concentrated smoke solutions for a short period and then transferring them to distilled water. It has also been found that the inhibitory effect, in most cases, can be remedied by rinsing the seeds (e.g. BROWN, 1993a), although in some cases smoke may be toxic to the seeds (e.g. KEELEY and FOTHERINGHAM, 1998a; also see **Section 2.3.2**). In terms of the post-fire environment, it has been speculated that if the smoke concentration in the soil is inhibitory immediately following a fire, germination will only occur once sufficient rainfall has fallen to dilute the solution, or wash away inhibitory compounds (BROWN, KOTZE and BOTHA, 1993; DE LANGE and BOUCHER, 1993b; LIGHT,GARDNER, JÄGER and VAN STADEN, 2002; also see **Section 4.3.3**).

2.2.7.2 Experimental considerations

In ***in situ* experiments**, inhibitory compounds present in smoke could be leached from the seeds or soil by irrigation or rainfall. However, in **laboratory germination studies**, inhibitory compounds would be retained in the Petri dish, and would have an influence on the final germination result (SPARG, KULKARNI and VAN STADEN, 2006). Furthermore, radical emergence is usually used as a measure of germination in laboratory studies. This often gives an estimate that is higher than when germination is measured as seedling emergence in seed trays (BROWN, 1993a). KEELEY and NITZBERG (1984) observed that the magnitude of charate-stimulated germination in

two chaparral species varied with medium and moisture levels. Importantly, they noted that slight differences, such as moisture level, in experimental conditions can produce subtle differences in germination. Similarly, KEELEY (1987) observed that the media used in germination trials had an effect on the germination of certain species. Some of the species tested germinated significantly better on soil, whereas other germinated better on filter paper. Thus, germination results obtained from Petri dish experiments cannot always be compared to those conducted in seed trays, or in the field.

The **viability of seed** samples is also an important consideration when evaluating germination treatments. For example, CROSTI, LADD, DIXON and PIOTTO (2006) selected 25 species typical of Mediterranean Basin plant communities for an investigation on the effect of aerosol smoke on these species. However, after evaluating the viability of these seeds (cut test method), only ten species had a viability level acceptable for experimentation (> 15% viability). Thus, in certain cases it is necessary to adjust the results accordingly to account for non-viable seeds (e.g. DIXON, ROCHE and PATE, 1995; ROCHE, DIXON and PATE, 1997; TIEU, DIXON, MENEY and SIVASITHAMPARAM, 2001a; PÉREZ-FERNÁNDEZ and RODRÍGUEZ-ECHEVERRÍA, 2003).

2.2.7.3 Physiological and environmental effects

From the numerous studies which have tested a wide variety of species for smoke-stimulated germination, it appears that the extent of influence of smoke on germination differs markedly within taxa and between seeds of different age or provenance (e.g. DIXON, ROCHE and PATE, 1995). These differences may be attributable to variations in **seed quality**, **seed dormancy patterns** and **seed longevity** (age and/or physiological state of the propagule). DE LANGE and BOUCHER (1993a) found that, following a smoke-tent treatment, only 13.3% of seeds in fresh fruits of *Audouinia capitata* germinated, whereas more than 80% of the oldest seeds germinated. Similarly, *Avena fatua* caryopses were more responsive to smoke solutions after a period of 8 or 12 weeks of after-ripening than freshly harvested caryopses, an observation that was particularly pronounced in the intact florets (ADKINS and PETERS, 2001).

Along with smoke, some species may also require additional factors to promote germination. The combined effects of **smoke and heat** are discussed further in **Section 2.2.8**. Some species require a period of **maturation** or **afer-ripening** in the soil, which may weaken the seed coat thereby allowing the smoke to enter the seed. This may be of particular importance in some cases where seed-coat imposed dormancy plays an important role.

In a study on 140 Australian native species, ROCHE, DIXON and PATE (1997) showed that some 25% species required both **soil storage** and smoke for maximal germination response. When the decline in viability was accounted for, this percentage was doubled (i.e. 55 species). KEELEY and FOTHERINGHAM (1998b) showed that for *Dendromecon rigida*, *Dicentra chrysantha*, and *Trichostema lanatum*, smoke treatments were ineffective unless coupled with a prior treatment of soil storage for 1 year. TIEU, DIXON, MENEY and SIVASITHAMPARAM (2001b) examined the interaction of **soil burial** and smoke on the germination patterns of eight Australian native species. Soil storage was not required for optimal germination of *Anigozanthos manglesii*, although for the other species tested, maximum germination was obtained only following a period of soil burial. Similarly, for seven of the eight fire ephemeral species studied, germination was enhanced following 12 months of soil burial, which was further enhanced by smoke or heat treatments (BAKER, STEADMAN, PLUMMER, MERRITT and DIXON, 2005b). KEELEY, McGINNIS and BOLLENS (2005) also found that, in general, soil storage increased the germination of controls and smoke (or heat) treatments for most of the chaparral species studied.

For *Actinotus leucocephalus* and *Tersonia cyathiflora*, two fire ephemerals from Western Australia, it was found that seeds of these species exhibited **dormancy cycling** during soil burial (BAKER, STEADMAN, PLUMMER, MERRIT and DIXON, 2005a). Seeds exhumed in spring showed lower levels of germination in response to smoke solution than seeds tested in autumn. Thus, long-term soil storage can alter the dormancy status of several species, resulting in changes in germination behaviour and responsiveness to smoke or heat treatments.

The effect of **light/darkness** is another factor that influences germination in species from fire-prone regions. In most cases, smoke-stimulated germination is not light dependent (KEELEY and FOTHERINGHAM, 1998b). However, for certain species, there does appear to be a significant interaction of smoke with light, requiring either light or darkness for germination. KEITH (1997), for example, found that in the endangered species *Epacris stuartii*, seeds germinated better in response to a smoke (or heat) treatment than in the light.

2.2.8 The interaction of smoke and heat

As mentioned in the previous section, the stimulatory effect of smoke may interact with heat and other fire-related cues. Numerous investigations, particularly by Australian researchers, have focussed on some of these interactive effects. The main findings of these studies are given in **Table 2.2**, which lists a number of studies on the independent effects of smoke and heat on germination, and **Table 2.3**, which lists several studies that have examined the combined effect of these factors.

Table 2.2: Examples of studies investigating the effects of smoke and heat, as independent treatments.

Publication title/Reference	Main findings
The independent effects of heat, smoke and ash on emergence of seedlings from the soil seed bank of a heathy *Eucalyptus* woodland in Grampians (Gariwerd) National Park, western Victoria ENRIGHT, GOLDBLUM, ATA and ASHTON, 1997	• Heat or smoke treatments of soil samples • Species richness and density greater for smoke or heat treated samples than control • 18 species (of 56) were found only in association with smoke or heat treatments
Germination and dormancy of grassy woodland and forest species: effects of smoke, heat, darkness and cold CLARKE, DAVISON and FULLOON, 2000	• Smoke stimulated germination in only 1 species (possibly due to the high concentration of smoke solutions) • Heat improved germination of 11 species (of 65)

Table 2.2 continued...

Publication title/Reference	Main findings
Smoke and heat effects on soil seed bank germination for the re-establishment of a native forest community in New South Wales READ, BELLAIRS, MULLIGAN and LAMB, 2000	• Smoke promoted an increase in density of species; grasses responded most strongly to the smoke (7 of 20 species) • Smoke and heat stimuli appeared to be complementary in promotion of seedling emergence (i.e. each treatment increased the density of different species)
Effects of smoke, heat and charred wood on the germination of dormant soil-stored seeds from a *Eucalyptus baxteri* heathy-woodland in Victoria, SE Australia ENRIGHT and KINTRUP, 2001	• Highest number of germinants and highest mean species richness was obtained for the smoke treatment • Heat treatments also improved density and species richness • The majority of species showed an enhanced germination response to both smoke and heat
Effects of heat and smoke on germination of soil-stored seed in a south-eastern Australian sand heathland WILLS and READ, 2002	• 55% of species in the germinable soil seed bank required smoke or heat to promote seed germination • Heat treatment resulted in highest mean species richness and seedling density (10x) • Smoke treatment also improved species richness and seedling density (5x)
Heat and smoke effects on the germination of seeds from soil seed banks across forest edges between subtropical rainforest and eucalypt forest at Lamington National Park, south-eastern Queensland, Australia TANG, BOULTER and KITCHING, 2003	• Found that smoke is an important germination trigger for species regenerating at the interface between subtropical and adjacent eucalypt-dominated wet sclerophyll forest
Seed germination of Sierra Nevada postfire chaparral species KEELEY, McGINNIS and BOLLENS, 2005	• Four species showed smoke-stimulated germination • Two species showed heat-stimulated germination • Two species were stimulated by both smoke and heat

Although the studies summarised in **Table 2.2** did not investigate any interactive effects of heat and smoke, they do illustrate the varied responses of different species to heat and smoke. For example, the treatment of a topsoil sample from a native forest community, with aerosol smoke or a heat treatment, showed that both treatments stimulated the germination of soil-stored seeds and appeared to be complementary in their promotion of seedling emergence. Each treatment increased the density of different species, enhanced the species richness of different components of the seed

bank, and had different effects on the rate of emergence (READ, BELLAIRS, MULLIGAN and LAMB, 2000). Similarly, ENRIGHT and KINTRUP (2001) found that, although the majority of species responded to both smoke and heat treatments, heat shock was a more specific requirement for triggering germination in hard-seeded species (e.g. Fabaceae), while smoke was effective on species from a broad range of other families.

In a study on the germination of eight species from the Sierra Nevada, it was observed that for four species, *Adenostoma fasciculatum*, *Eriodictyon crassifloium*, *Mentzelia dispersa* and *Mimulus bolanderi*, germination was significantly enhanced in the treatments with smoke solutions, but not by any of the heat treatments. In contrast, *Fremontodendron californicum* and *Malacothamnus fremontii* had significantly higher germination in the heat treatments, but not for any of the smoke treatments (KEELEY, McGINNIS and BOLLENS, 2005). In the case of soil-stored seeds of *Eriodictyon crassifloium* and *Mentzelia dispersa*, germination was significantly increased by both smoke and heat, whereas in fresh seed only the smoke treatment resulted in a significant improvement (KEELEY, McGINNIS and BOLLENS, 2005).

Table 2.3: Examples of studies investigating the interactive effects of smoke and heat.

Publication title/Reference	Main findings
Combined effects of heat shock, smoke and darkness on germination of *Epacris stuartii* Stapf., an endangered fire-prone Australian shrub KEITH, 1997	• In the dark, heat or smoke improved germination of *E. stuartii* • Combined treatment of heat and smoke (in the dark) resulted in greater germination than either treatment alone - additive effect
Ecological aspects of soil seed-banks in relation to bauxite mining. I. Unmined jarrah forest WARD, KOCH and GRANT, 1997	• 19 species showed a response to heat treatment • 13 species responded negatively to heat • Only one species responded significantly to smoke
Ecological aspects of soil seed-banks in relation to bauxite mining.II. Twelve year old rehabilitated mines GRANT and KOCH, 1997	• 13 species (of 70) responded to smoke • 11 species (of 70) responded to heat • Majority of species responded to either smoke or heat treatment, rather than combined treatment

Table 2.3 continued...

Publication title/Reference	Main findings
Heat shock, smoke and darkness: partner cues in promoting seed germination in *Epacris tasmanica* (Epacridaceae) GILMOUR, CROWDEN and KOUTOULIS, 2000	• In the dark, heat (but not smoke) slightly improved germination of *E. tasmanica* • Combined treatment of heat and smoke (in the dark) resulted in greater germination than heat alone - synergistic effect • Smoke and heat treatment also improved germination in other *Epacris* species
Influence of multiple fire-related germination cues on three Sydney *Grevillea* (Proteaceae) species KENNY, 2000	• For *G. buxifolia* a synergisitc effect between heat and smoke was observed (i.e. combined effect greater than germination obtained with either treatment alone) • For *G. sericea* smoke improved germination, and an additive effect observed for smoke and heat treatment • For *G. speciosa* germination with smoke or heat treatment significant, but smoke gave greater improvement
Germination response of seven east Australian *Grevillea* species (Proteaceae) to smoke, heat exposure and scarification MORRIS, 2000	• Smoke improved germination in all seven species tested • Heat improved germination in four species • For *G. buxifolia*, *G. diffusa*, *G. juniperina*, *G. linearifolia* and *G. speciosa* smoke and heat combined had an additive effect
Seed coat dormancy in two species of *Grevillea* (Proteaceae) MORRIS, TIEU and DIXON, 2000	• Heat or smoke treatment improved germination of *G. linearifolia*; no additive effect observed
The interaction of heat and smoke in the release of seed dormancy in seven species from southwestern Western Australia TIEU, DIXON, MENEY and SIVASITHAMPARAM, 2001a	• Heat alone, and with smoke, promoted germination of five species • Three species responded to treatment of smoke only • *Stylidium affine* showed a strong response to smoke, regardless of heat treatment • For *Sowerbaea laxiflora*, smoke alone, or combination of heat and smoke promoted germination (not heat alone)
Spatial and developmental variation in seed dormancy characteristics in the fire-responsive species *Anigozanthos manglesii* (Haemodoraceae) from Western Australia TIEU, DIXON, MENEY, SIVASITHAMPARAM and BARRETT, 2001	• Tested variability of response to smoke and/or heat in different populations • For most populations, seed showed significant response to smoke treatment • Combined treatment of smoke and heat further increased germination

Table 2.3 continued...

Publication title/Reference	Main findings
Interactive effects of heat shock and smoke on germination of nine species forming soil seed banks within the Sydney region THOMAS, MORRIS and AULD, 2003	• Two species did not respond to any treatments • *Gahnia sieberiana* and *Kunzea ambigua* responded independently to smoke or heat, and additively to combined treatment • *Kunzea capitata* showed a synergistic response (heat alone gave no response, but increased the response to smoke) • *Baeckea diosmifolia* and *Baeckea imbricata* responded only to combined treatment - unitive effect
Fire-related cues break seed dormancy of six legumes of tropical eucalypt savannas in north-eastern Australia WILLIAMS, CONGDON, GRICE and CLARKE, 2003b	• Tested 10 tropical savanna legumes • Six species germinated in response to heat treatments • Smoke had no effect alone or with heat treatment
Dormancy release in Australian fire ephemeral seeds during burial increases germination response to smoke water or heat BAKER, STEADMAN, PLUMMER, MERRITT and DIXON, 2005b	• For *Actinotus leucocephalus* and *Codonocarpus cotinifolius* a combined heat and smoke treatment resulted in higher germination than either treatment alone • Four species responded to smoke treatments (germination enhanced by soil burial)
Germination response to heat and smoke of 22 Poaceae species from grassy woodlands CLARKE and FRENCH, 2005	• For six species (of 22), no response to heat or smoke observed • Heat influenced germination of five species • Smoke influenced germination of six species • For six species, effect of smoke varied with temperature
The effect of fire-related germination cues on the germination of a declining forest understorey species TIERNEY, 2006	• For *Prostanthera askania*, smoke improved germination whereas heat treatment had little or no effect and reduced the effect of smoke treatment

From **Table 2.3** it can be seen that in a number of species the effects of smoke and heat on germination have been shown to be interactive. The **different types of responses** to smoke and/or heat can be described as follows:

(1) Independent - where seeds germinate in response to either a heat shock treatment, or smoke treatment (e.g. GRANT and KOCH, 1997; GILMOUR, CROWDEN and KOUTOULIS, 2000);

(2) Independent and additive - where seed germination in response to a combined

treatment is greater than either treatment alone (e.g. KEITH, 1997; KENNY, 2000; MORRIS, 2000; THOMAS, MORRIS and AULD, 2003);

(3) Synergistic - where the response to a combined heat and smoke treatment is greater than the addition of the effects of both treatments applied alone (e.g. GILMOUR, CROWDEN and KOUTOULIS, 2000; KENNY, 2000; THOMAS, MORRIS and AULD, 2003); or

(4) Unitive - where a germination increase occurs only in response to a combined heat and smoke treatment (e.g. GILMOUR, CROWDEN and KOUTOULIS, 2000; MORRIS, 2000; THOMAS, MORRIS and AULD, 2003).

For several species the **interaction** of certain levels of heat and smoke have provided the **best germination response**. Examples of this include *Actinotus leucocephalus* (TIEU, DIXON, MENEY and SIVASITHAMPARAM, 2001a), *Gahnia sieberiana* and *Kunzea ambigua* (THOMAS, MORRIS and AULD, 2003). A study on three *Grevillea* species found that both smoke and heat increased the germination, although the relationship between the two treatments varied with species (KENNY, 2000). Similarly, MORRIS (2000) also found that smoke and heat treatments combined had an additive effect for four *Grevillea* species from east Australia. The investigation by TIEU, DIXON, MENEY and SIVASITHAMPARAM (2001a) on the interaction of heat and smoke on the germination of seven Australian species also resulted in significant germination with a combined treatment for some species.

CLARKE and FRENCH (2005) examined the effects of heat and smoke on 22 members of the Poaceae. For 5 species, a significant interaction was found. Of these, 2 species showed an **additive effect** of heat and smoke, where the application of both enhanced the germination more than either factor alone. In the case of *Epacris tasmanica*, a **synergistic effect** was observed. Although treatment with a smoke solution did not show any germination improvement, it raised germination levels to 49% when combined with a heat treatment (heat treatment alone gave 12.3%) (GILMOUR, CROWDEN, KOUTOULIS, 2000).

The effect of smoke and heat on germination of seeds of *Anigozanthos manglesii* was

shown to be variable, **depending on population** (TIEU, DIXON, MENEY, SIVASITHAMPARAM and BARRET, 2001). In general, heat stimulated germination significantly more than the aerosol smoke treatment, although there was a further increase in germination when the smoke and heat treatments were combined.

The study by THOMAS, MORRIS and AULD (2003) highlighted the importance of using a **range of heat shock levels**, since some species respond better to low-intensity heat treatments (e.g. *Baeckea imbricata*). Additionally, this study illustrates the importance of testing the interactive effects of heat and smoke, which together promote the germination of species which otherwise may be overlooked (e.g. *Baeckea* sp.).

Although GRANT and KOCH (1997) found that the majority of species responded to either a smoke or heat treatment, rather than to the combined treatment, these studies indicate that the germination of seeds from fire-prone regions may be linked to **multiple fire-related cues**. TIEU, DIXON, MENEY and SIVASITHAMPARAM (2001a) concluded that the variations in the interactions between heat and smoke treatments suggest that germination in the post-fire environment will occur over a range of conditions, both spatially and temporally, thereby facilitating seedling survival and limiting competition between emerging species. Thus, heat and smoke may play an interactive role in breaking dormancy and in germination under natural conditions, although it is apparently a complex and variable process. Interestingly, however, some species such as *Stylidium affine* exhibit a **strong reaction to smoke**, where no heat treatment is as effective in breaking seed dormancy as smoke (TIEU, DIXON, MENEY and SIVASITHAMPARAM, 2001a). Thus, for this species, recruitment is highly linked to smoke as a germination cue.

2.3 SOURCES OF SMOKE

2.3.1 Chemical components of smoke

The **analysis of chemicals** present in smoke have largely been as a result of the use of smoke and smoke condensates in the food industry (see **Section 2.3.3.1**), and

research on cigarette smoke (MAGA, 1988). Emissions from burning vegetation, wood, or other organic material, is a highly complex system consisting of a suspension of particulate matter (ash and soot), droplets and vapours (gases). The gases produced include large amounts of carbon dioxide (CO_2), high concentrations of carbon monoxide (CO), ammonia, ethylene (C_2H_4), nitrogen oxides and sulphur dioxide (IMANISHI and FORTANIER, 1982/83; UYEMURA and IMANISHI, 1983).

Smoke and liquid smoke (smoke condensates) also contain numerous other compounds including: organic acids; organic bases; alcohols; aldehydes; esters; alkyl aryl ethers; furan and pyran derivatives having alcoholic, ketonic, and aldehydic functional groups; ketones and diketones; lactones; phenolic derivatives; guaiacol derivatives, syringol derivatives; hydrocarbons; and some nitrogenated derivatives (SAKUMA, MUNAKATA and SUGAWARA, 1981; MAGA, 1988; GUILLÉN, MANZANOS and ZABALA, 1995; GUILLÉN and IBARGOITIA, 1996; ANDREOLI, GIGANTE and NUNZIATA, 2003). It is now known that cigarette smoke, and smoke from other products, contains about **4800 compounds** (ANDREOLI, GIGANTE and NUNZIATA, 2003).

The fuel type (nature of the wood), particle size, moisture content, temperature of the process and available oxygen during the process all influence the **relative composition** of the compounds formed (SAKUMA, MUNAKATA and SUGAWARA, 1981). This is of particular interest to food chemists interested in the flavour and aroma chemistry related to food smoking (MAGA, 1988; GUILLÉN and IBARGOITIA, 1996; GUILLÉN and IBARGOITIA, 1999). Importantly, many of the **hydrocarbons** found in smoke include polycyclic aromatic hydrocarbons (PAHs) and related polynuclear aromatic hydrocarbons (PNAHs) which are toxic or carcinogenic. For example, benzene, 1,3-butadiene, benzo[*a*]pyrene and 4-(methylnitrosamino)-1-butanone, known carcinogens, are found in cigarette smoke and other types of smoke (ANDREOLI, GIGANTE and NUNZIATA, 2003).

2.3.2 Methods of smoke treatments

Various methods have been used to treat seeds with smoke. In general, the stimuli in

smoke are transferred to seeds in 3 ways: (1) direct exposure to aerosol smoke; (2) indirect exposure through smoked growth media; and (3) irrigation or soaking with smoke-saturated water.

2.3.2.1 Aerosol smoke and smoked media

The occurrence of wildfires during dry seasons in many parts of the world results in large volumes of smoke being released into the atmosphere. As discussed above, aerosol (airborne) smoke contains particulate matter, water vapour, volatile gases and numerous other compounds. Other (non-volatile compounds) may become trapped in water droplets, or adhere to other particles and are "swept up" with the smoke by the air currents as a result of convection from the fire. These **smoke clouds** can drift for many miles, sometimes affecting plant communities a great distance away from the original fire site (PARMETER and UHRENHOLDT, 1975). As a result, such smoke may potentially impact environments outside the immediate vicinity of the fire, either directly as aerosol smoke, or via aqueous media, be it moist soil or **water runoff** into watercourses. PRESTON and BALDWIN (1999), for example, found evidence for the transport of the germination cue by wind and water into adjacent unburned areas (from 40 m to 1 km away from a burned site).

A number of researchers investigating smoke-stimulated seed germination have used aerosol smoke to treat seeds *in situ* or *ex situ*. The general method involves generating smoke in a drum and then directing the smoke into a polyethylene tent. The **cooled smoke** is allowed to settle on the soil in open plots, or the seeds to be treated are sown in trays and placed inside the smoke-filled tent for the duration of the treatment. DE LANGE and BOUCHER (1993a) demonstrated that smoke-stimulated seed germination of *Audouinia capitata* was independent of soil depth in the range of 5-30 mm, indicating that the smoke is able to penetrate the soil, or is leached into the soil by rainfall. **Table 2.4** gives examples of different aerosol smoke treatments that have been used in various studies (also referred to as "dry smoking"). From the various examples in the table it can be seen that a wide variety of fuel sources (plant material) have been used.

Table 2.4: Examples of smoke production for aerosol smoke treatments.

Preparation	Fuel	Reference
Smoke generated in a 130 L drum and passed through a cooling pipe into plastic tents (0.5 x 0.5 m plots) for 30 min	Mix of dry and fresh fynbos vegetation	DE LANGE and BOUCHER, 1990
Pots placed in plastic tent; smoke generated in a 130 L drum and passed through a cooling pipe into tent for 30 min; pots left in smoke tent for 2 h; irrigated with distilled water	Mix of dry and green leaf and stem material of fynbos shrub *Passerina vulgaris*	BROWN, 1993a BROWN, 1993b BROWN, KOTZE and BOTHA, 1993
Smoke generated in a bee-keepers smoker and introduced at base of a wooden chest (80 x 50 x 40 cm) with a loose fitting lid; different exposure times tested (5, 15, 45 and 90 min); dry or pre-imbibed seeds smoked	Leaf material of *Themeda triandra*	BAXTER, VAN STADEN, GRANGER and BROWN, 1994
Smoke generated from 50 g dried material in a bee-keepers smoker and introduced into a plastic bag containing Petri dishes with seeds for 3 min; sealed for a further 2 min; filter paper moistened with distilled water	Leaf material from 27 different montane grass species	BAXTER, GRANGER and VAN STADEN, 1995
5 g of material burned and smoke bubbled through 50 mL distilled water; tested at different dilutions	Tissue paper; leaves of *Acacia mearnsii*, *Eucalyptus grandis*, *Hypoxis colchicifolia*, *Pinus patula*	JÄGER, LIGHT and VAN STADEN, 1996
10 g of material combusted on a hot plate and funnelled into a 70 L chamber containing dry seed; treated for 1 or 10 min	*Adenostoma fasciculatum* foliage	KEELEY and FOTHERINGHAM, 1997
Smoke from a 60 L steel combustion drum passed through a cooling pipe into plastic tents (1.5 x 1.5 x 1 m) for 60 min	Mix of dry and green, leaf and fine stem material from jarrah forest	ROCHE, KOCH and DIXON, 1997
Material heated in a 150 mm metal pan on a hot plate; glass funnel placed upside down and smoke directed through 1 cm x 50 cm long rubber hose into 70 L chamber; smoke pulled into tank with vacuum line	Small branches and leaves of *Adenostoma*	KEELEY and FOTHERINGHAM, 1998b
Seeds placed in sieves atop a 1.5 m chimney above smouldering grass; 30, 60 and 90 min exposure times; rinsed following treatment	Semi-dry *Heteropogon contortus*	SPARG, KULKARNI and VAN STADEN, 2006

In some studies, seeds are first sown in trays/pots containing soil or potting mix, and the **trays/pots are infused with aerosol smoke** inside a plastic tent, followed by a light

irrigation (e.g. BROWN 1993a). Alternatively, they may be gently watered before treatment with aerosol smoke (e.g. CROSTI, LADD, DIXON and PIOTTO, 2006). Other studies have employed a method of **"direct smoking" of seeds**, where the seeds are openly exposed to aerosol smoke, before being placed in germination media (e.g. BAXTER, VAN STADEN, GRANGER and BROWN, 1994; SPARG, KULKARNI, LIGHT and VAN STADEN, 2005). In a study on the effect of smoke on *Themeda triandra*, it was observed that the germination response to aerosol smoke increased with an increased state of imbibition (BAXTER, VAN STADEN, GRANGER and BROWN, 1994). Similarly, SPARG, KULKARNI and VAN STADEN (2006), in a study on commercial maize, first soaked maize kernels in water prior to direct exposure to aerosol smoke.

Smoke can also be applied to media such as activated clays or sand particles (DIXON and ROCHE, 1995). KEELEY and FOTHERINGHAM (1998b) tested the aqueous transfer of smoke by sowing untreated seeds directly into **smoke-treated soil**, or onto **smoked filter paper**. Interestingly, KEELEY and FOTHERINGHAM (1998b) found that seeds of *Emmenanthe penduliflora* and *Romneya coulteri* germinated when exposed indirectly to vapours emitted by moistened smoke-treated sand or filter paper.

The **optimal exposure time** to smoke treatments differs between species and also depends on the method used. KEELEY and FOTHERINGHAM (1997) observed that seeds of *Emmenanthe penduliflora* tolerated only 10 min of direct exposure to smoke. In a study on woodland species from central-western Spain, some Asteraceae and Fabaceae species showed the highest germination response after 10 min of smoke exposure, whereas *Cistus* species required a longer application (20 min) to reach higher germination levels (PÉREZ-FERNÁNDEZ and RODRÍGUEZ-ECHEVERRÍA, 2003).

2.3.2.2 Aqueous smoke solutions

The use of aerosol smoke for improving seed germination is fairly cumbersome, and potentially dangerous. Furthermore, the many variables involved in smoke production make it difficult to produce identical batches of smoke. DE LANGE and BOUCHER (1990) illustrated that the active component(s) of aerosol smoke are water soluble and

that a **smoke-saturated solution** was effective at improving germination. The general method involves generating smoke in a drum by the slow combustion of plant material (see **Figure 3.1, Section 3.2.1**). The heavy acrid smoke is then directed into a container of water, or bubbled through a "water-trap" using compressed air. **Table 2.5** gives a summary of different examples of preparation of smoke solutions.

Table 2.5: Examples of smoke extract production for smoke water treatments.

Preparation	Fuel	Reference
Smoke generated in 18 L drum, bubbled through distilled water using compressed air	Mix of dry and fresh fynbos vegetation	DE LANGE and BOUCHER, 1990
Smoke generated in 18 L drum, bubbled through 2.5 L distilled water for 30 min using compressed air; range of dilutions tested	Mix of dry and green leaf and stem material of fynbos shrub *Passerina vulgaris*	BROWN, 1993a BROWN, 1993b BROWN, JAMIESON and BOTHA, 1994
Smoke bubbled through 500 mL of water for 45 min; range of dilutions tested	Leaf material of *Themeda triandra*	BAXTER, VAN STADEN, GRANGER and BROWN, 1994
Smoke from a 60 L steel combustion drum bubbled through 100 L of water for 60 min	Mix of dry and green, leaf and fine stem material from jarrah forest	ROCHE, KOCH and DIXON, 1997
Smoke generated in 18 L metal drum and drawn through 20 L water for 1 h; sterilised by autoclaving at 121 °C and 100 kPa for 20 min	6 kg of fresh and dry foliage of *Eucalyptus*, *Adenanthos* and *Banksia*	TIEU, DIXON, SIVASITHAMPARAM, PLUMMER and SIELER, 1999

In the early stages of research on smoke-stimulated germination, a critical question was whether the **type of plant material** burned influenced the germination response (BROWN, 1993a; BAXTER, GRANGER and VAN STADEN, 1995). BAXTER, VAN STADEN, GRANGER and BROWN (1994) showed that smoke extracts derived from fynbos material or burnt *Themeda triandra* promoted the germination of seeds of *T. triandra*. In a further study, smoke was produced from the leaf material of 27 montane grassland species, 26 of which improved the germination of *T. triandra* seeds (BAXTER, GRANGER and VAN STADEN, 1995). In a further study by JÄGER, LIGHT and VAN STADEN (1996), it was shown that smoke from a variety of source materials, including laboratory tissue paper, was effective at promoting germination of light-sensitive Grand Rapids lettuce seeds in the dark. Hence, it appears that smoke produced from burning

any type of plant material can effectively promote germination of smoke-responsive species (also see **Section 7.1.1**).

As discussed in **Section 2.2.7**, the concentration of a smoke solution is an important consideration. Highly concentrated smoke extracts inhibit germination, but are not necessarily toxic to the seeds. Certainly, not all species respond equally, and they appear to have differential sensitivity to compounds in the smoke solutions. Thus, a non-response could be indicative of a too high, or too low, concentration of smoke solution (BROWN and VAN STADEN, 1997).

Concentrated smoke extracts are usually very **acidic** (pH 2-4), and it has been suggested that the inhibitory effects observed with smoke may be due to the acidic conditions of smoke solutions (BALDWIN, STASZAK-KOZINSKI and DAVIDSON, 1994). Dilutions of smoke extracts, however, are much less acidic, and thus less likely to have any significant effect on germination. KEELEY and FOTHERINGHAM (1998a), noted that smoke solutions buffered to pH > 7 were less effective at breaking dormancy in *Emmenanthe penduliflora*, although for this species, brief pulses of acids were also effective in breaking dormancy. In general, however, the effectiveness of smoke solutions in breaking seed dormancy does not appear to be dependent on the pH of the smoke extract, and buffered solutions are equally effective at breaking dormancy (BROWN and VAN STADEN, 1997; TODOROVIĆ, GIBA, ŽIVKOVIĆ, GRUBIŠIĆ and KONJEVIĆ, 2005).

Importantly, the activity of these smoke solutions is not reduced after **autoclaving** (JÄGER, STRYDOM and VAN STADEN, 1996; TIEU, DIXON, SIVASITHAMPARAM, PLUMMER and SIELER, 1999). Thus, smoke solutions could also be used in sterile plant cultures. Furthermore, there appears to be no loss in activity of some smoke solutions which have been stored for over 10 years in containers that have repeatedly been opened (see **Section 3.2.1**).

2.3.3 Commercial smoke extracts

2.3.3.1 Smoke in the food industry

Exposure of foods to wood smoke is one of the oldest means of **preserving foods** such as fish and meat. The heat and dry air partially dehydrate the food during the smoking process, thereby reducing microbial growth. In addition, the absorbed smoke contains chemicals which render antioxidant properties, inhibit microbial contamination, as well as flavouring the food (LUSTRE and ISSENBERG, 1969; SINK, 1979). **Section 2.5.1** gives more information on the antimicrobial properties of smoke.

In current commercial practice, natural wood smoking has mostly been replaced by the use of **liquid smoke flavourings**, and is employed primarily for the colour and added aroma and flavour (SOFOS, MAGA and BOYLE, 1988). Liquid smoke was developed in the 1970s for use in the food industry because of concern regarding the presence of potentially carcinogenic benzopyrenes, and for convenience of use (HOLLEY and PATEL, 2005). The use of these smoke condensates provides a method for avoiding the contamination of smoked foods by polycyclic aromatic hydrocarbons (PAHs). However, some studies have shown the presence of PAHs in commercial smoke products (GOMAA, GRAY, RABIE, LOPEZ-BOTE and BOOREN, 1993; YABIKU, MARTINS and TAKAHASHI, 1993), although GUILLÉN, MANZANOS and ZABALA (1995) were unable to detect PAHs in their analysis of a liquid smoke flavouring.

It is also of interest that liquid food-flavouring smoke has been shown to promote seed germination (BALDWIN, STASZAK-KOZINSKI and DAVIDSON, 1994). JÄGER, RABE and VAN STADEN (1996) investigated the effects of two commercial food flavourants produced in South Africa on the germination of Grand Rapids lettuce seed. Both extracts showed inhibition of germination at high concentrations (1:1000 dilution and above), but promoted germination at lower concentrations (dilutions in the region of 1:100 000). Similarly, DOHERTY and COHN (2000) used a commercial liquid smoke to break the dormancy of *Oryza sativa.*

2.3.3.2 Commercial smoke products for seeds

Since smoke solutions effectively promote the germination of many species from a wide range of families, the commercial development of smoke treatments for the stimulation of seed germination is a viable option. Smoke and smoke solutions have the potential for a variety of applications in agriculture, horticulture and land restoration (see **Section 2.6**). To date, a number of commercially available smoke products have been developed, and are listed in **Table 2.6**. Although liquid smoke preparations are highly effective, the active components in smoke can also be applied via sand or filter paper previously exposed to smoke (BROWN, 1994; ENRIGHT, GOLDBLUM, ATA and ASHTON, 1997; KEELEY and FOTHERINGHAM, 1997).

Table 2.6: Commercial smoke products available for use in improving germination.

Trade name	Comments	Reference
Fire Grow™	100 mL smoke water extract per L of distilled water Unistel Technologies (Pty) Ltd, South Africa	BEKAARDT, PIETERSE, COETZEE and AGENBAG, 2004
Kimseed Seed Germination Smoke Tent	Aerosol smoke treatment tent Kimseed Pty Ltd, Australia	www.kimseed.com.au (Accessed on 3 May 2004)
Regen 2000® Germinator	A granulated smoke product for use with seed tray applications, on a nursery level; Australia	www.tecnica.com.au/regen%20germinator.html (Accessed on 3 May 2004)
Regen 2000® Direct	A highly concentrated smoke water designed to be applied directly to broad-acreage soil by boom or aerial spraying; Australia	www.tecnica.com.au/regen%20direct.html (Accessed on 3 May 2004)
Regen 2000® Seedstarter	A highly concentrated smoked vermiculite designed for broad acreage applications; Australia	www.tecnica.com.au/regen%20seedstarter.html (Accessed on 3 May 2004)
Regen 2000® Smokemaster	A liquid smoke extract for use in a horticultural environment and for the treatment of seeds prior to broadcast seeding operations; Australia	www.tecnica.com.au/regen%20smokemaster.html (Accessed on 3 May 2004)
Seed Starter®, Australian Smoky Water	Smoked water solution Dilute according to instructions Kings Park and Botanical Gardens, Perth, WA, Australia	ADKINS, DAVIDSON, MATTHEW, NAVIE, WILLS, TAYLOR and BELLAIRS, 2000 ADKINS and PETERS, 2001

Table 2.6 continued...

Trade name	Comments	Reference
Kirstenbosch Instant Smoke Plus (Super Smoke Plus Cape Seed Primer)	Smoke infused filter paper; germination promoters (gibberellins) are included as an additive Water is added to the paper and seeds are soaked in the solution National Botanical Institute at Kirstenbosch, Cape Town, South Africa (also distributed by Cape Seed and Book Suppliers, South Africa)	BROWN, 1994 BROWN and VAN STADEN, 1997 www.seedman.com/cape.htm www.freshestseeds.com/cape.html www.nbi.ac.za/research/hortresearchseedprimer.htm (Accessed 3 May 2004)

The effective concentration range of the smoke water may depend on the preparation procedures. There are a number of factors that could influence the concentration of the biologically active components in the production of smoke solutions. These include factors such as the design of the apparatus, the moisture status and type of fuel used, the temperature and rate of combustion and the duration of exposure to the solute (BAXTER, GRANGER and VAN STADEN, 1995; BOUCHER and MEETS, 2004).

ADKINS and PETERS (2001) tested different commercial smoke solutions on seeds of *Avena fatua* and *Malva neglecta*, and found differences in the optimal dilution for germination between the different solutions. Thus, for commercial preparation of smoke solutions, it is of obvious concern that the products are standardised and are able to elicit the required germination response at the given dosage.

2.4 MODE OF ACTION OF SMOKE-STIMULATED GERMINATION

Germination is generally defined as the commencement of water uptake by imbibition of the dry seed, followed by embryo expansion. This usually results in the rupture of the covering layers and radicle protrusion, which is regarded as the completion of the germination process (KUCERA, COHN and LEUBNER-METZGER, 2005). In most germination studies, a seed is considered to have germinated upon the visible emergence of the radicle.

In one of the first in-depth studies on smoke-stimulated seed germination, BALDWIN, STASZAK-KOZINSKI and DAVIDSON (1994) proposed three classes of possible

mechanisms for smoke-stimulated seed germination: (1) **chemical scarification** of the seed coat; (2) **nutritive stimulation** of germination; and (3) **signal-mediated stimulation** of germination. There have been several studies which have attempted to clearly define the mechanism of smoke-stimulated seed germination, and these are discussed below. However, at present, the site and mode of physiological action of smoke-stimulated germination is still unknown, although it is known that smoke does contain a highly active chemical cue (see **Chapter 6**; FLEMATTI, GHISALBERTI, DIXON and TRENGOVE, 2004; VAN STADEN, JÄGER, LIGHT and BURGER, 2004).

2.4.1 Scarification vs a signal-mediated mechanism

As mentioned above, BALDWIN, STASZAK-KOZINSKI and DAVIDSON (1994), proposed three possible classes for the mode of action of smoke in promoting germination. In experiments aimed at resolving the mode of action, no visible damage to the seed coat of *Nicotiana attenuata* seeds was observed when treated with smoke solutions. Additionally, **abrasion of the seed coat**, and treatments to quench organic oxidants, did not stimulate germination. Thus, it was concluded that smoke solutions do not promote germination via a mechanism involving scarification of the seed coat. Furthermore, no difference in biomass was observed between seeds germinated with smoke extracts or hypochlorite after 7 days, indicating that it was unlikely that smoke functioned via a nutritive stimulation. Interestingly, in seeds of *N. attenuata* with scarified seed coats, treated with a smoke solution for 72 h and soaked in 1% tetrazolium dye, the embryo and endosperm turned bright red. In contrast, in seeds treated with KNO_3, the seeds remained pale. These results indicated that the smoke solution stimulated metabolic activity in the seeds, thus supporting the hypothesis of a "signal-mediated" mechanism for smoke-stimulated germination (BALDWIN, STASZAK-KOZINSKI and DAVIDSON, 1994).

EGERTON-WARBURTON (1998), found that exposing seeds of *Emmenanthe penduliflora* to aerosol smoke resulted in major changes to the morphology of the seed. The study, based on transmission and scanning electron microscopic analysis, showed that the **seed-coat layers** were destabilised by the smoke treatment. External changes

to the seed surface, similar to chemical scarification, were observed. Additionally, smoke significantly altered the permeability of the internal (sub-testa) cuticle, as demonstrated with the **fluorescent apoplastic tracer dye**, lucifer yellow carbohydrazide (LY-CH). In untreated seeds, LY-CH was restricted to the periphery of the seed, whereas following a smoke treatment, LY-CH was observed throughout the endosperm and embryo. However, water uptake rates were similar between control and smoke-treated seeds of *E. penduliflora*. Thus, EGERTON-WARBURTON (1998) concluded that the causal factors for smoke-stimulated germination in *E. penduliflora* were a combination of increased cuticular permeability and a concomitant leaching of endogenous inhibitors during imbibition.

KEELEY and FOTHERINGHAM (1997) observed a similar pattern of **eosin and LY-CH dye** in *Emmenanthe penduliflora*. In control seeds, the dyes did not penetrate beyond the subdermal cuticle, but following treatments with either smoke or NO_2, they were found in the endosperm and embryo. Likewise, KEELEY and FOTHERINGHAM (1998b) observed the same for seeds of *Emmenanthe*, *Phacelia grandiflora*, *Romneya*, and *Dicentra*. In these species, eosin dye readily permeated the endosperm and embryo following smoke treatment. In *Camissonia* seeds, dye penetration was blocked by an outer cuticle, but following a smoke treatment, complete dye penetration was observed. Interestingly, in seeds of *Eucrypta*, dye permeated through the endosperm of dormant seeds, but was blocked from entry into the embyro. Following smoke treatment, however, the dye was found throughout the seed. These differences in the types of barriers to germination, led to the suggestion by KEELEY and FOTHERINGHAM (1998b) that the mechanism of smoke-induced germination may be different between smoke-induced species. Although smoke treatments appear to increase the solute permeability of the semi-permeable subdermal membrane, it is unknown whether this directly influences the induction of germination.

As mentioned previously, smoke contains various organic acids, and smoke solutions are very acidic (see **Section 2.3.1** and **Section 2.3.2.2**). Thus, it is not unlikely that these acidic compounds are responsible for the chemical scarification and cuticular breakdown observed in some seeds. Certainly, this may play a role in promoting

germination, but is not the only mechanism at work in smoke-stimulated seed germination. KEELEY and FOTHERINGHAM (1998b) comment that it is possible that the mechanism behind scarification-induced germination is unrelated to smoke-stimulated germination. Although smoke can apparently alter the external features of seeds, there is more evidence to suggest that smoke acts primarily as a chemical cue, and therefore acts internally in the seed. The isolation of a highly active germination promoter from plant-derived smoke confirms this, and given the low concentration of the compound necessary to elicit a response in seeds of many species it is likely that an alternative mechanism is operational (see **Section 6.3.2**).

2.4.2 Stimulatory chemicals in smoke

It has been suggested that the germination activity of smoke is due to **ammonia** or **ethylene** (VAN DE VENTER and ESTERHUIZEN, 1988) or **octanoic acid** (SUTCLIFFE and WHITEHEAD, 1995), but studies using Grand Rapids lettuce seeds have shown that neither of these compounds can account for the promotive effect of smoke solutions on the germination of these lettuce seeds (VAN STADEN, DREWES and BROWN, 1995; JÄGER, STRYDOM and VAN STADEN, 1996). Furthermore, exogenous applications of ethrel or gaseous ethylene did not stimulate the germination of *Themeda triandra* seeds, which do respond to smoke treatments, suggesting that ethylene is not the active component of smoke (BAXTER, VAN STADEN, GRANGER and BROWN, 1994). Additionally, KEELEY and FOTHERINGHAM (1998b) found that ethylene did not stimulate germination in any of the chaparral species which responded significantly to smoke treatments.

KEELEY and FOTHERINGHAM (1997, 1998a) have suggested that a likely active component in smoke is **nitrogen oxide**. However, in a recent study by PRESTON, BECKER and BALDWIN (2004) it was also concluded that nitrogen oxides are not components of smoke that elicit germination in two smoke-stimulated species, *Nicotiana attenuata* and *Emmenanthe penduliflora*. **Chapter 5** discusses the role of nitric oxide in smoke-stimulated germination in greater detail.

Current research clearly indicates the presence of a highly active germination compound in plant-derived smoke (see **Chapter 6**; FLEMATTI, GHISALBERTI, DIXON and TRENGOVE, 2004; VAN STADEN, JÄGER, LIGHT and BURGER, 2004). It is not unlikely, however, that other chemicals in smoke may also play some role as chemical cues in the environment.

2.4.3 The interaction of smoke and plant growth regulators

There is considerable evidence that the basic mechanisms of dormancy and seed germination are primarily controlled by the plant hormones **gibberellins (GAs)** and **abscisic acid (ABA)** (KARSSEN, ZAGÓRSKI, KĘPCZYŃSKI and GROOT, 1989; THOMAS, 1992; DERKX, VERMEER and KARSSEN, 1994; KOORNNEEF, BENTSINK and HILHORST, 2002). Gibberellic acid (GA_3) is known to control hydrolysis of storage materials in the endosperm of seeds, thereby facilitating mobilisation of food reserves and reducing mechanical constraints imposed on the embryo (GROOT and KARSSEN, 1987; HILLHORST and KARSSEN, 1992). In contrast, ABA acts as a suppressor of germination, and is known to induce dormancy (HILLHORST and KARSSEN, 1992).

Since plant hormones play a critical role in the germination of seeds, a number of studies on smoke-stimulated seed germination have also investigated possible **interactions of smoke with plant growth regulators** in order to gain some insight into the mode of action of smoke. A summary of such studies is given in **Table 2.7**.

Additionally, several studies have been conducted using light-sensitive Grand Rapids lettuce seeds to investigate physiological aspects of smoke-stimulated seed germination. These are discussed in greater detail in **Chapter 3** (DREWES, SMITH and VAN STADEN, 1995; VAN STADEN, JÄGER and STRYDOM, 1995; JÄGER, STRYDOM and VAN STADEN, 1996; STRYDOM, JÄGER and VAN STADEN 1996).

Table 2.7: Summary of studies on smoke in combination with plant growth regulators. (ABA = abscisic acid; ACC = 1-aminocyclopropane-1-carboxylic acid; BA = N^6-benzyladenine; GA_3 = gibberellic acid; $GA_{4/7}$ = GA_4 and GA_7 gibberellin mixture; R = red light; SM = smoke solution).

Species/Reference	Comments and main findings
Lactuca sativa cv. Grand Rapids (lettuce) see **Chapter 3**	• Phytochrome-controlled seed germination • Thermodormancy can be induced • SM increases sensitivity to GAs and ABA
Themeda triandra BAXTER, VAN STADEN, GRANGER and BROWN, 1994	• No interactive effect between ethylene and SM
Syncarpha vestita BROWN and VAN STADEN, 1994	• Negatively photoblastic seeds • Responsive to $GA_{4/7}$ or $GA_{4/7}$ + BA • BA alone had no effect • Responsive to SM • SM/$GA_{4/7}$ more effective
Apium graveolens (celery) THOMAS and VAN STADEN, 1995	• High and low dormancy cultivars tested • For dormant cultivar, $GA_{4/7}$ or SM alone did not promote germination in the dark • Combination of SM with $GA_{4/7}$ overcame dormancy in the dark (more effective at 18 °C than at 26 °C) • For low dormancy cultivar, $GA_{4/7}$ alone promoted germination in the dark (no additive effect in combination with SM)
Apium graveolens (celery) THOMAS and VAN STADEN, 1995	• SM alone slightly increased the germination of low dormancy cultivar • In the absence of $GA_{4/7}$, SM, BA and ethephon were ineffective • SM enhanced partial dormancy-breaking effect of 10 min R • Suggested that SM acts similarly to cytokinins by enhancing GA activity
Actinotus leucocephalus TIEU, DIXON, SIVASITHAMPARAM, PLUMMER and SIELER, 1999	• SM, or GA_3 + zeatin not effective in alleviating dormancy in whole seeds • SM, or GA_3 + zeatin significantly promoted germination of decoated seeds • No additive effect observed with SM, GA_3 and zeatin
Stylidium affine TIEU, DIXON, SIVASITHAMPARAM, PLUMMER and SIELER, 1999	• SM improved germination of whole seeds • GA_3 + zeatin only slight improvement in whole seeds, more effective in decoated seeds • Some synergistic effect observed with SM, GA_3 and zeatin in decoated seeds
Calluna vulgaris (heather) THOMAS and DAVIES, 2002	• Seeds are photodormant and thermodormant (depth of dormancy can vary considerably) • $GA_{4/7}$ effectively breaks dormancy • SM and $GA_{4/7}$ combined more effective than SM alone

Table 2.7 continued...

Species/Reference	Comments and main findings
Nicotiana attenuata KROCK, SCHMIDT, HERTWECK and BALDWIN, 2002 SCHWACHTJE and BALDWIN, 2004	• SM or $GA_{1,3,4,7}$ increases germination of dormant seeds • Paclobutrazol inhibited germination of SM-treated seeds • SM increases sensitivity to exogenous GA_3 in light and dark; light increases sensitivity ten-fold • Fluridone (ABA synthesis inhibitor) with SM resulted in a greater germination increase than SM alone
Paulownia tomentosa (Empress tree) TODOROVIĆ, GIBA, ŽIVKOVIĆ, GRUBIŠIĆ and KONJEVIĆ, 2005	• Phytochrome-controlled seed germination • SM could not induce germination in the dark • SM-imbibed seeds, with R pulse, induced high germination if SM present during imbibition phase or phase of phytochrome activity • GAs completely substituted light requirement • In combination with SM, lower concentrations of GAs were required for maximum germination • SM allowed light-induced germination when applied with germination retardants - Paclobutrazol, ancymidol or tetcyclacis (in combination, or after retardant treatment)
Avena fatua (wild oat) KĘPCZYŃSKI, BIAŁECKA, LIGHT and VAN STADEN, 2006	• SM increased the germination of both intact seeds and caryopses • GA_3 increased the germination of caryopses (not intact seeds) • Combination of GA_3 and SM increased the germination rate • Although SM increased germination, it did not result in changes in α-amylase activity • GA_3 and ethylene involved in regulation of α-amylase activity during germination • Combination of SM and ACC resulted in higher ethylene levels than either treatment alone

From these investigations, it seems that the overall effect of smoke, in combination with exogenously applied hormones, is to sensitize seeds to the hormones, particularly GAs (VAN STADEN, BROWN, JÄGER and JOHNSON, 2000). Thus, there clearly appears to be some **involvement of smoke with GAs**, although in some instances there also appears to be some interaction with other plant hormones (also see **Section 3.3.3**).

Smoke treatments can effectively promote germination in seeds of some species, in which dormancy is also broken by GA application. For example, germination of dormant seeds of *Syncarpha vestita* has been induced using $GA_{4/7}$, $GA_{4/7}$ with BA or smoke (BROWN, KOTZE and BOTHA, 1993). ADKINS and PETERS (2001) found that smoke solutions could overcome dormancy of a good proportion of freshly harvested caryopses

of *Avena fatua*. As such, its effect was similar to that of GA_3, which was highly effective at promoting germination of the caropyses.

In a study of treatments for the release of dormancy of heather (*Calluna vulgaris*) seeds, THOMAS and DAVIES (2002) showed that soaking seeds for 4 h in a mixture of smoke solution and $GA_{4/7}$ improved the speed and percentage germination by at least ten-fold. Treating the seeds with a smoke solution or $GA_{4/7}$ improved germination above the controls, but was more effective when used in combination. Similarly, TODOROVIĆ, GIBA, ŽIVKOVIĆ, GRUBIŠIĆ and KONJEVIĆ (2005) observed that treating seeds of *Paulownia tomentosa* with a combination of exogenously applied GAs and smoke solution reduced the effective concentration of GAs, indicating an increased sensitivity to the applied GAs.

It is important to note, however, that smoke is not always able to substitute for the action of exogenous GAs in breaking dormancy and promoting germination. Seeds of two cultivars of dormant celery (*Apium graveolens* L.) were use to investigate the relationship between smoke, $GA_{4/7}$, BA and red-light on dormancy break (THOMAS and VAN STADEN, 1995). In the absence of $GA_{4/7}$, the smoke extract had little promotory effect, but in combination dormancy was partially broken. Thus, from these results it was suggested that smoke acts in a similar way to cytokinins, by enhancing GA activity.

For seeds of *Helichrysum aureonitens*, GA_3 improved germination to 45% (in comparison to the control, which gave 29% germination (AFOLAYAN, MEYER and LEEUWNER, 1997). A smoke treatment did not improve germination, and instead showed signs of inhibition, resulting in a germination level of 10%. Likewise, in *Hibbertia amplexicaulis* (Dillenaceae), GA_3 treatments, following scarification, significantly improved germination to 55%, whereas treatment with a smoke solution did not improve germination levels above that of the scarified control seeds (ALLAN, ADKINS, PRESTON and BELLAIRS, 2004). Thus, in these species, the smoke treatment appeared to be ineffective at substituting for GA. It is possible, however, that the smoke treatments used in these studies were not optimal for these species. The decrease in germination observed in *H. aureonitens* suggests that the concentration may have been too high (see **Section 2.2.6**).

A few studies have investigated the effects of **growth retardants** on the action of smoke-stimulated seed germination (also see **Section 3.3.3**). KEELEY and FOTHERINGHAM (1998a) applied chlorocholine chloride (CCC), an inhibitor of GA biosynthesis, to scarified or smoke-treated seeds of *Emmenanthe penduliflora*. The CCC was highly inhibitory to scarified seeds, but not to smoke-treated seeds, indicating that scarification and smoke may act through different mechanisms. In contrast, however, treatment of light-sensitive Grand Rapids lettuce seeds with the GA biosynthesis inhibitors paclobutrazol or AMO 1618 resulted in an inhibition of germination (GARDNER, DALLING, LIGHT, JÄGER and VAN STADEN, 2001; see **Section 3.3.3**). Likewise, in *Nicotiana attenuata*, the application of paclobutrazol inhibited germination in non-dormant seeds, as well as in smoke-treated dormant seeds (KROCK, SCHMIDT, HERTWECK and BALDWIN, 2002). SCHWACHTJE and BALDWIN (2004) also found that paclobutrazol, at a concentration of 10 µM, completely inhibited germination of *N. attenuata* when applied up to 12 h after the smoke treatment (1 h soak in smoke solution). However, paclobutrazol may also have an effect on ABA degradation which could result in low germination levels (KROCK, SCHMIDT, HERTWECK and BALDWIN, 2002).

2.4.4 Possible modes of action

As discussed in the previous section, smoke seems to sensitize the seeds to exogenous plant hormones. In addition, smoke may also have an **effect on the phytochrome** system of certain seeds. In light-sensitive Grand Rapids lettuce seeds, smoke solutions are able to overcome the light requirement, thereby enhancing germination in the dark (see **Chapter 3**). Conversely, negatively photoblastic seeds of *Syncarpha vestita* are able to germinate in the light when treated with a smoke solution (BROWN, 1993b).

Importantly, however, smoke is not always able to substitute for the light requirement of seeds. For example, smoke solutions were ineffective in promoting the germination of light-sensitive seeds of *Paulownia tomentosa* in the dark (TODOROVIĆ, GIBA, ŽIVKOVIĆ, GRUBIŠIĆ and KONJEVIĆ, 2005). In *Shoenia filifolia* subsp. *subulifolia*, although no significant germination response was observed with smoke treatments, GA_3

stimulated germination in both dark and light, following 3 months of post-harvest storage (PLUMMER, ROGERS, TURNER and BELL, 2001). After 27 weeks of dry storage, however, the exposure of seeds to a smoke solution was able to replace the requirement of light in the germination process, illustrating that the physiological state of the seed can play an important role in its response to smoke treatment. Furthermore, it also indicates that smoke and GA do not necessarily function in the same manner.

Some possible suggestions of how smoke may act as a chemical germination cue have been discussed in VAN STADEN, BROWN, JÄGER and JOHNSON (2000). **Table 2.8** gives a summary of these hypotheses.

Table 2.8: Possible modes of action of smoke-stimulated seed germination as discussed in VAN STADEN, BROWN, JÄGER and JOHNSON (2000).

Possible mode of action	Argument
Activation of the phytochrome system	Some seeds that are not under phytochrome control can also be promoted to germinate by smoke, thus the phytochrome system is unlikely to be the direct target
Interaction with plant hormone receptors, thereby changing their sensitivity	It seems unlikely that smoke would be able to change the sensitivity of all the receptors of different plant hormones (cytokinins, gibberellins, ethylene and ABA)
Influence the biosynthesis/metabolism of gibberellins and/or other plant hormones, thus increasing the amount of plant hormones that can respond to the phytochrome system	Some species of Restionaceae, which cannot be germinated using conventional methods (including treatments with plant growth regulators), respond to smoke treatments (BROWN, JAMIESON and BOTHA, 1994) suggesting that smoke does not act via plant hormones
Promotion of changes in membrane permeability, thereby enhancing transport of plant hormones to active sites	This could explain the increased sensitivity to different plant hormones
Activation of enzymes crucial to the initiation of reserve mobilization and the commencement of germination	A more likely possibility, given the fact that smoke also appears to influence growth rate (e.g. BLANK and YOUNG, 1998; see **Section 2.5.5**), and that many different types of seeds respond to smoke treatments

Few studies have investigated, in detail, the effect of smoke on endogenous levels of plant hormones (also see **Section 3.3.3** for studies on Grand Rapids lettuce). However, KROCK, SCHMIDT, HERTWECK and BALDWIN (2002) and SCHWACHTJE and BALDWIN (2004) have examined the effects of smoke on the dormancy and endogenous GA and ABA pools in seeds of ***Nicotiana attenuata***.

KROCK, SCHMIDT, HERTWECK and BALDWIN (2002) found that both smoke and exogenously applied GA_3 were effective in breaking the dormancy of *N.attenuata* seeds, whilst the GA biosynthesis inhibitor paclobutrazol inhibited germination in smoke-treated seeds. Thus, it was suggested that smoke induces GA biosynthesis (KROCK, SCHMIDT, HERTWECK and BALDWIN, 2002). In the same study, endogenous ABA was measured in seeds of *N. attenuata* treated with smoke or fluridone, an inhibitor of ABA biosynthesis. Although no significant differences were observed in the ABA content between the two treatments, the fluridone-treated seeds germinated earlier than the smoke-treated seeds (KROCK, SCHMIDT, HERTWECK and BALDWIN, 2002).

In a further study on smoke-stimulated seed germination of *N. attenuata*, SCHWACHTJE and BALDWIN (2004) analysed the endogenous GA and ABA levels of smoke-treated seeds using GC-MS. Analyses were performed during the 22 h after the start of imbibition, when smoke-treated seeds commit to germination. The extractable GA_{1+3} pools showed a decrease in both the control and smoke-treated seeds. However, in the smoke-treated seeds, a greater decrease was observed within 2 h of smoke exposure. This was followed by a slight increase between 2 and 4 h, and a gradual decrease thereafter. Levels of ABA in smoke-treated seeds showed a small initial increase after 2 h, followed by a dramatic decrease. In the control seeds, however, the ABA increased after the start of imbibition and thereafter remained stable. These results suggest that the reduction of ABA is necessary to initiate germination, a notion consistent with the prior germination results obtained with fluridone-treated seeds (KROCK, SCHMIDT, HERTWECK and BALDWIN, 2002; SCHWACHTJE and BALDWIN, 2004).

It was also found that, in the dark, treatment of dormant seeds of *N. attenuata* with smoke did not promote germination, although GA_3 treatment did (SCHWACHTJE and

BALDWIN, 2004). Furthermore, in comparison to the germination of seed treated only with GA_3, smoke significantly enhanced the germination of seeds treated with lower concentrations of GA_3 (10 and 100 µM). Exposure of the seeds to light increased this sensitivity an additional 10-fold (i.e. seeds treated with smoke and 1 µM GA_3 did not germinate in the dark, but did so in the light). Thus, as described above, there seems to be some possible involvement of the phytochrome system in smoke-stimulated germination of *N. attenuata* seeds (SCHWACHTJE and BALDWIN, 2004).

Taken together, these results indicate that smoke treatments increase GA sensitivity, and are correlated with a decrease in endogenous ABA pools (SCHWACHTJE and BALDWIN, 2004). Furthermore, the results suggest that smoke promotes *de novo* GA_3 synthesis, which is possibly linked to light exposure and thus phytochrome-mediated. TODOROVIĆ, GIBA, ŽIVKOVIĆ, GRUBIŠIĆ and KONJEVIĆ (2005) also concluded that smoke may have an effect on the rate of GA biosynthesis. Through the use of different growth retardants in combination with smoke solutions, it was observed that the required concentration of growth retardant to reduce germination of *Paulownia tomentosa* was higher when applied simultaneously with smoke solution, than when applied alone.

Studies, to date, on the mechanism of smoke-stimulated seed germination have used crude smoke solutions, containing a complex mixture of thousands of compounds. The isolation of a highly active compound from plant-derived smoke (described in **Chapter 6**; FLEMATTI, GHISALBERTI, DIXON and TRENGOVE, 2004; VAN STADEN, JÄGER, LIGHT and BURGER, 2004) will now allow for more focussed research into the mode of action, without the additional effects of other compounds present in smoke.

2.5 ADDITIONAL BIOLOGICAL RESPONSES TO SMOKE

Apart from the effect of smoke on seed germination, there are a number of other biological responses to smoke. These include: antimicrobial and antifungal activities; dormancy-breaking in bulbs; stimulation of flowering; and improvements of seedling vigour, root development and somatic embryogenesis.

2.5.1 Antimicrobial activity

As mentioned earlier in **Section 2.3.3.1**, the process of smoking food reduces antimicrobial contamination, and has been widely used as a method of food preservation. A number of studies have illustrated the **antibacterial effect** of food smoke preparations, and HOLLEY and PATEL (2005) have recently reviewed the antimicrobial activity of liquid smoke used in the food industry. In general, the antimicrobial activity of liquid smoke has been attributed to the presence of carbonyls, organic acids and phenols in liquid smoke (VITT, HIMELBLOOM and CRAPO, 2001), although among other considerations, the food composition is also an important factor for the overall antimicrobial activity of liquid smoke preparations (SUÑEN, FERNANDEZ-GALIAN and ARISTIMUÑO, 2001).

Examples of studies on the antibacterial activity of smoke include a study by ERDMAN, WATTS and ELLIAS (1954) on the effect of liquid smoke on cultures of *Bacillus subtilis*, *Proteus vulgaris* and *Staphylococcus aureus*. This study showed some inhibition and reduction in growth of all these organisms at different dilutions of the liquid smoke. More recently, SOFOS, MAGA and BOYLE (1988) tested the ether extracts of smoke condensates from 20 different wood types against three strains of *Aeromonas hydrophila* and three strains of *Staphylococcus aureus*. Results from this study showed variation in inhibition of growth depending on the type of liquid smoke used, and the species and strain of microorganism tested. ASITA and CAMPBELL (1990) investigated the effect of smoke extracts from seven different woods against *Escherichia coli*, *S. aureus* and *Saccharomyces cerevisiae*. The ethanolic extracts exhibited varying degrees of antimicrobial against these organisms, probably due to differences in composition of the various smoke extracts. Three extracts, however, inhibited growth of all the test organisms at concentrations of 20 mg mL^{-1}.

2.5.2 Antifungal activity

PARMETER and UHRENHOLDT (1975) investigated the effect of smoke on **spore germination and mycelial growth** of several fungi. Germination of conidia on

cellophane exposed to smoke reduced the spore germination of five species of fungi, namely *Botrytis gemella*, *Fomes annosus*, *Fusarium lateritium*, *Penicillium expansum* and *Peridium harknessii*. For these species, the degree of sensitivity varied, and in some cases greatly reduced conidial germination. For *Trichoderma* sp., an increase in germination for smoke exposure times less than 16 min was observed. Mycelial growth of *F. annosus*, *Pholiota adiposa*, *Trichosporium symbioticum* and *Verticicladiella wagenerii* was reduced on cellophane exposed to smoke. Bean and pine seedlings were also exposed to smoke prior to inoculation with bean rust fungus and western gall rust fungus, respectively. The exposure markedly reduced the rust infection, indicating that smoke deposits on plant surfaces were inhibitory to fungal infection.

2.5.3 Dormancy-breaking in bulbs

IMANISHI and FORTANIER (1982/83) have investigated the possibility of shortening the high temperature **dormancy-breaking treatment** of freesia corms by exposure to ethylene or smoke. The promotive effect on sprouting by smoke was slightly less than that of **ethylene** (5 µL L^{-1}, applied over 2 days), which strongly promoted sprouting of the corms and substituted for 2 weeks of high temperature treatment. However, there was no, or only a slight, difference in average flowering time between ethylene and smoke-treated corms and the controls. The response to smoke treatment in this case may be due to the ethylene which was present in the aerosol smoke at between 56 and 77 µL L^{-1}. It is interesting to note that the promotive effects of ethylene diminished with increasing exposure period. In a related study, UYEMURA and IMANISHI (1983) examined the effect of a number of hydrocarbons, and other gases present in smoke, on the dormancy in freesia corms. Ethylene, at levels as low as 1 µL L^{-1}, strongly stimulated dormancy release of the corms.

IMANISHI (1983) has also investigated the effects of **smoke and ethylene treatments** on the flowering (due to dormancy breaking) of *Narcissus tazetta* bulbs. Smoke treatment, as practised by a grower, with smouldering wood and fresh leaves for several hours on ten consecutive days, resulted in a higher flowering percentage 1-2 weeks earlier than the controls. A smoke treatment of 4 days was sufficient to effectively

promote flowering, and longer treatments slightly delayed flowering. In addition to releasing dormancy, it is also possible that smoke confers other benefits such as antimicrobial properties, as discussed in **Section 2.5.1**.

2.5.4 Flowering

Fire-stimulated flowering is common in many monocotyledons and has been reported in Amaryllidaceae, Cyperaceae, Iridaceae, Liliaceae, Orchidaceae and Poaceae. The exact nature of the stimulus varies and can be attributed to ethylene in the smoke, changes in temperature, light and nutrients (BOND and VAN WILGEN, 1996). Evidence from various studies supports the hypothesis that fire-stimulated flowering is not as a result of direct effects of fire, but rather by indirect effects of fire on the microclimate, such as altered soil temperature regimes (LE MAITRE and BROWN, 1992).

In South Africa, the majority of the terrestrial orchids flower irrespective of fires. However, a number of species flower in response to wildfires (SCHELPE, 1970; HOLMES, 1986). SCHELPE (1970) separates these **fire-responsive orchids** into two groups. The first group flower prolifically following a fire and thereafter the production of inflorescences diminishes in years subsequent to the occurrence of the fire. Examples include *Bartholina burmanniana*, *Ceratandra atrata*, some *Disa* species, *Neobolusia tysonii*, *Orthopenthea bivalvata* and the two species of *Penthea* (SCHELPE, 1970). The second group, however, appear to be fire-dependent and flower only in the year subsequent to a fire. Species which have only been observed to flower following a fire include several *Corycium* species, *Disa salteri*, *Evota bicolor, E. harveyana*, *Forficaria graminifolia*, *Pachites bodkinii* and *Satyridium rostratum* (SCHELPE, 1970; HOLMES, 1986). To date, however, there is not much known about the factors which are involved in stimulating the flowering of these orchids.

The **fire-lily** *Cyrtanthus ventricosus* (Amaryllidaceae) is a fynbos geophyte whose flowering has been observed to be associated with fire (OLIVIER and WERNER, 1980; LE MAITRE and BROWN, 1992). It has been hypothesised that this fire-related flowering response is due to various gases, such as ethylene, as observed in pineapples

(TRAUB, COOPER and REECE, 1940; TOMPSETT, 1985). KEELEY (1993), however, has shown that flowering in *C. ventricosus* can be induced with smoke, and not ethylene. Cool aerosol smoke was applied to potted *C. ventricosus* plants in a smoke-filled chamber for 24 h. In a second treatment, plants were incubated under 100 ppm ethylene for 48 h. Within 3 days, each of the bulbs from the smoke treatment developed a flowering stalk whereas the plants from the control and ethylene treatment failed to flower, even after one month.

2.5.5 Seedling vigour

Findings from various studies have shown that the effects of smoke can extend beyond germination. It has been suggested that a more rapid time to emergence and improved seedling growth may significantly enhance the survival of seedlings which are able to compete more effectively with undesirable species in a post-fire environment (BROWN and VAN STADEN, 1997; READ, BELLAIRS, MULLIGAN and LAMB, 2000).

BAXTER and VAN STADEN (1994) observed a **faster rate of germination** of smoke-treated seeds of *Themeda triandra*, which also grew more vigourously than untreated seeds. A similar effect was also seen in some *Erica* species and species of Asteraceae (BROWN, 1993a). Likewise, BLANK and YOUNG (1998) observed that exposing seeds of some common species of the sagebrush-steppe of the western United States to smoke significantly increased the seedling growth rate relative to controls. Similarly, although final germination values were similar, a more **rapid emergence** of some species from the Mediterranean Basin was observed following an aerosol smoke treatment (CROSTI, LADD, DIXON and PIOTTO, 2006).

In some cases, an **enhancement in seedling length or mass**, in comparison to controls, has been observed. For example, the growth of oilseed rape (*Brassica napus*) seedlings was greatly enhanced with a solution of a pyrolised wood extract (THORNTON, THOMAS and PETERS, 1999). SPARG, KULKARNI, LIGHT and VAN STADEN (2005) investigated the effect of aerosol smoke and smoke solutions on the germination and seedling vigour of three South African medicinal plants. In general, no

stimulatory effect on the final percentage germination was observed. Although there was no effect on the final germination percentage, two of the three species examined (*Albuca pachychlamys* and *Tulbaghia violacea*) showed improved seedling vigour.

Although research on smoke-stimulated seed germination has received increased attention in the last decade, the use of fire and smoke in agriculture is not a new practice. Farmers have traditionally used smoke in grain drying practices, a method which is believed to improve germination and seedling vigour (PAASONEN, HANNUKKALA, RÄMÖ, HAAPALA and HIETANIEMI, 2003). In South Africa, some rural farmers store maize cobs over a fireplace, thus subjecting the seeds to smoke and heat. MODI (2002, 2004) has investigated the effect of this storage method on the seed quality of two traditional maize landraces. The method was found to improve the germination rate and final germination in comparison to untreated seeds. Furthermore, seeds stored above the fireplace produced significantly more vigorous seedlings that were heavier and taller than untreated seeds. In a study on a commercial maize cultivar, SPARG, KULKARNI and VAN STADEN (2006) found that various treatments with aerosol smoke and smoke solutions resulted in enhanced germination and a significant improvement of seedling vigour.

2.5.6 Root development

Improved seedling vigour, as discussed above, may be attributable to **increased root growth**. TAYLOR and VAN STADEN (1996) investigated the effect of smoke solutions on root initiation in mung bean (*Vigna radiata*) cuttings, using an assay originally developed by HESS (1964) for the detection of substances, such as indoleacetic acid (IAA) and indolebutyric acid (IBA), which stimulate root initiation. Treatments with smoke solutions significantly increased the number of roots, in comparison to the control. The optimal dilutions for the smoke solutions, derived from burnt *Themeda triandra* or *Passerina vulgaris*, were 1:50 and 1:25, respectively. The highest concentrations of the *Themeda* smoke (1:5 and 1:10 dilution) were detrimental to the cuttings, resulting in wilting and rotting. Results from this study also showed that the immersion time was an important factor for root initiation. For the *Themeda* smoke

solution, shorter pulses (4 h and 8 h) resulted in significantly more roots than the 16 h pulse treatment, at dilutions of 1:100 and 1:50.

Following the above-mentioned study, TAYLOR and VAN STADEN (1998) also investigated the effect of smoke solutions on the growth of *in vitro* tomato (*Lycopersicon esculentum*) root cultures. Both the *Themeda* and *Passerina* smoke solutions significantly promoted the extension of the primary root axis and increased the **frequency of lateral roots** at dilutions of 1:1500 and 1:2000. No significant differences for the average length of the secondary roots was observed. Fractionation of the crude smoke solutions by thin layer chromatography (TLC) revealed that several of the fractions significantly promoted primary root growth, indicating the possibility of more than one active compound. The promotive effect of the smoke solutions on secondary root frequency was, however, reduced following TLC fractionation, suggesting a synergistic effect of the compounds in the smoke solutions.

2.5.7 Somatic embryogenesis

SENARATNA, DIXON, BUNN and TOUCHELL (1999) investigated the effects of smoke solutions on the development of **somatic embryos** of geranium (*Pelargonium hortorum*) hypocotyl cultures. Using a three phase system (explant, induction, expression) the effect of a 10% smoke solution on somatic embryogenesis was evaluated. The addition of smoke solution at either the explant or the induction stage produced the highest number of somatic embryos and enhanced the rate of embryo development. In the best treatment (smoke exposure at the induction phase), 70% of all the embryos were at a cotyledonary stage at day 25 of the experiment, in comparison to 15% in the untreated controls. Importantly, a prolonged exposure to the smoke solution was detrimental.

2.6 POTENTIAL APPLICATIONS OF SMOKE TECHNOLOGY

Despite the fact that the identity of the active compound(s) was unknown for a long time, and that the mode of action is not yet fully understood, smoke and smoke solutions can be utilized for a variety of applications. Examples of their current use include

horticulture, agriculture and habitat restoration. As described in **Section 2.3.3.2**, there are already a number of commercially available smoke products for use in improving germination. There exists, however, even wider opportunities for the use of smoke and smoke solutions in horticulture and agriculture, as well as the potential use of smoke as seed pre-treatments, and for weed control and ecological studies.

2.6.1 Horticulture

In recent years **indigenous gardening** has become very popular in South Africa. Unfortunately, many fynbos species are difficult to germinate and cannot easily be propagated by members of the public. The use of smoke technology, however, can be used to promote the germination of many of these plants for horticultural purposes and for sale to garden enthusiasts. As discussed in **Section 2.2.2**, there are now more than 150 fynbos species that exhibit a good germination response to smoke. BROWN and BOTHA (2004) have recently published a review on the seed germination studies of plants from the major families of the Cape Floristic Region. Included in this, are protocols which can be used as a general guide in obtaining optimum germination for species in each family.

Of particular importance has been the dormancy breaking of many of the Restionaceae species. These important fynbos species have great potential for **suburban landscaping**. Prior to the use of smoke for promoting seed germination, many of the approximately 300 species of Restionaceae were difficult or impossible to propagate from seed. Many of these species, however, respond well to smoke treatments and are now more easily available for horticultural use. Smoke treatments enable a greater efficiency in germinating seeds and thus more plants are obtained from a seed sample. Additionally, the range of fynbos species available for cultivation is increased (BROWN, JAMIESON and BOTHA, 1994; JAMIESON and BROWN, 1995; BROWN and BOTHA, 2004).

The poor germination of many attractive indigenous species has limited their use in **floriculture**. Thus, smoke treatments may increase the propagation and commercial

development of such species. Furthermore, smoke may also have uses for propagating species of importance to the **wildflower industry**, such as *Syncarpha vestita* (Cape everlasting) which is widely used as a dried flower. Increased cultivation of these species is important in terms of conservation, since it would reduce the pressure on wild populations as a result of collecting (BROWN, 1993a).

Researchers at Kirstenbosch Botanical Gardens have developed a **seed primer** which incorporates aqueous smoke extracts and a mixture of other natural germination stimulators. The dehydrated seed primer is impregnated onto absorbent paper which can be used by gardeners. The product is marketed as "Kirstenbosch Instant Smoke Plus Seed Primer" (BROWN, 1994). Likewise, researchers at King's Park and Botanic Gardens, in Australia, have also developed a similar commercial product which is marketed as "Seed Starter® ". It is a concentrated smoke solution which is used in a diluted form (also see **Section 2.3.3.2**).

2.6.2 Agriculture

As discussed in **Section 2.5.5**, several studies have shown that smoke treatments enhance the rate of germination and improve **seedling vigour** of a variety of agricultural plants. RAZANAMANDRANTO, TIGABU, SAWADOGO and ODÉN (2005) demonstrated that smoke treatments significantly shortened the mean germination time for some savanna-woodland species with both non-dormant and dormant seeds. Some vegetable crops, such as lettuce (*Lactuca sativa* L. cv. Grand Rapids) (DREWES, SMITH and VAN STADEN, 1995) and celery (*Apium graveolens* L.) (THOMAS and VAN STADEN, 1995), have shown enhanced germination with smoke. Thus, smoke treatments could possibly be used to promote synchronous germination of seeds, and to increase the rate of germination of certain agricultural crops.

2.6.3 Smoke as a primer or pre-treatment

BALDWIN, STASZAK-KOZINSKI and DAVIDSON (1994) observed that once *Nicotiana attenuata* seeds were soaked in a smoke solution for more than 15 min, it was not

possible to prevent germination by rinsing the seeds. Similarly, BROWN (1993a) found that flushing seeds of *Syncarpha vestita* treated with a 1:2 dilution of a smoke extract relieved the inhibitory effect of the relatively high concentration of the smoke solution, but did not reduce the stimulatory effect of the smoke. It is this **"retention" of the germination cue**, once seeds have been exposed to smoke, that allows for the pre-treatment and subsequent storage of seeds prior to sowing, and has important implications for the use of smoke in horticulture, agriculture and in ecological rehabilitation (ROCHE, DIXON and PATE, 1997; BROWN and VAN STADEN, 1998; TIEU, DIXON, SIVASITHAMPARAM, PLUMMER and SIELER, 1999).

BAXTER and VAN STADEN (1994) **pre-treated seeds** of *Themeda triandra* by imbibing them in a diluted smoke solution, allowing the seeds to dry under a stream of air and then storing the seeds at 25 °C for a period of 21 days. For *T. triandra*, a smoke pre-treatment period of 12 h was most effective in promoting germination. The promotive effect of the smoke on germination was retained after this storage period, and when re-imbibed the seeds germinated as well as smoke-treated seeds that were germinated immediately. Additionally, no detrimental effect on subsequent seedling growth was observed with the pre-treated seeds.

Seeds of *Syncarpha vestita* and *Rhodocoma gigantea* were pre-treated in a smoke solution for 24 h before being dried and stored at 18 °C (BROWN, PROSCH and BOTHA, 1998). Seed germination was assessed monthly, by germinating the seeds under conditions previously found to be suitable for fynbos species. For both species, the significant dormancy-breaking effect of the smoke treatment was retained up to and including 12 months of storage. For *S. vestita*, the control seeds showed slightly variable germination during the 12 months, with some loss in dormancy over time. The smoke pre-treated seed showed significant promotion of germination, with a slight increase in dormancy break over time. For *R. gigantea*, the control seeds retained a high level of dormancy, and although the smoke pre-treated seeds were significantly improved, this did decrease slightly over the assessment period. Nonetheless, these results do indicate the effectiveness and long-lasting effect of the smoke treatments.

DIXON and ROCHE (1995) also pre-treated seeds of several Australian species prior to sowing. Seeds were treated with aerosol smoke in a fumigation tent for 60 min, or were soaked for 12 h in a smoke solution and dried and stored until required. TIEU, DIXON, SIVASITHAMPARAM, PLUMMER and SIELER (1999) also tested four species from Western Australia. The species tested, namely *Actinotus leucocephalus*, *Conostylis setigera*, *Sylidium affine* and *Stylidium brunonianum*, have important horticultural potential although their germination is unreliable. Seeds of these species were soaked for differing durations in smoke solutions. After soaking, the seeds were rinsed, dried and stored for 3 weeks. Although seeds of all the species tested were able to be pre-treated, the optimum imbibition times did vary.

From these studies it appears that pre-treatment with smoke is a feasible method for treating seeds. In particular, this process lends itself to handling larger quantities of seed for horticultural and agricultural purposes (BROWN and VAN STADEN, 1997). Care should be taken, however, since seeds of some species can degenerate if soaked for prolonged periods, in which case pre-treatment could lead to a decline in seed quality and viability. Thus, there is a need to test seeds of each species for tolerance to imbibition and drying in order to optimize pre-treatment conditions (DIXON and ROCHE, 1995).

2.6.4 Weed control

Many weed species have the ability to persist in the soil seed bank for several years due to seed dormancy. This attribute often hampers the task of predicting the timing and the extent of weed emergence for **weed management** in agricultural systems (ADKINS, DAVIDSON, MATTHEW, NAVIE, WILLS, TAYLOR and BELLAIRS, 2000; BENECH-ARNOLD, SÁNCHEZ, FORCELLA, KRUK and GHERSA, 2000). Since many weed species respond favourably to smoke treatments (see **Section 2.2.6**), it is possible to better evaluate the extent to which a soil seed bank is contaminated by weed seeds, and also assess the seed reserve of indigenous species (GRANT and KOCH, 1997; VAN STADEN, BROWN, JÄGER and JOHNSON, 2000).

Smoke technology may be used in agricultural management to reduce the weed burden

on crops and decrease the need for herbicides or physical weed control. Furthermore, it may even be possible to deplete arable weed seed banks through appropriate smoke application (ADKINS, DAVIDSON, MATTHEW, NAVIE, WILLS, TAYLOR and BELLAIRS, 2000; ADKINS and PETERS, 2001). For example, smoke treatments could be used to manage weed populations of arable land by breaking dormancy thereby maximising seedling emergence. Following this, other mechanisms could be used for weed eradication (BENECH-ARNOLD, SÁNCHEZ, FORCELLA, KRUK and GHERSA, 2000). However, this aspect of smoke stimulated seed germination of weedy species requires more research, particularly in agricultural ecosystems (ADKINS, PETERS, PATERSON and NAVIE, 2003).

2.6.5 Ecological studies using smoke

A **seed bank** is the term given to a reserve of viable, ungerminated seeds in a habitat (BASKIN and BASKIN, 1998). THOMPSON and GRIME (1979) define two general types of seed banks: transient and persistent. In a transient seed bank, none of the seeds produced in a given year remain viable in the habitat for more than a year, whereas seeds in a persistent seed bank remain dormant for longer periods. WALCK, BASKIN and BASKIN (1996) have modified this definition slightly and suggest that the two types of seed banks should be defined in terms of germination seasons. Following this reasoning, a transient seed bank is composed of seeds that do not survive until the second germination season following maturation. A persistent seed bank is composed of seeds that survive until the second (or more) germination season(s).

BALDWIN, STASZAK-KOZINSKI and DAVIDSON (1994) were among the first to suggest the possible use of smoke as an ecological tool for the study of dormant seed banks, and showed that treating artificial seed banks with smoke solutions resulted in significant increases in the number of germinating seedlings. It was also shown that the germination potential of smoke-treated soil could be retained for at least 53 days. Many of the more recent studies involving smoke-stimulated seed germination have investigated the size and spatial extent of soil seed banks (ENRIGHT, GOLDBLUM, ATA and ASHTON, 1997; WARD, KOCH, and GRANT, 1997; LLOYD, DIXON and SIVASITHAMPARAM, 2000; READ, BELLAIRS, MULLIGAN and LAMB, 2000;

ROKICH, DIXON, SIVASITHAMPARAM and MENEY, 2002; TANG, BOULTER and KITCHING, 2003). By promoting seed germination with smoke, the assessment of soil seed bank samples is greatly enhanced, and species which require fire-related germination cues are less likely to be overlooked (TANG, BOULTER and KITCHING, 2003). Thus, smoke can act as a surrogate for fire in studies aimed at understanding the dynamics of fire season on recruitment and survival of species (DIXON and BARRETT, 2003).

In a study conducted over two growing seasons in a Western Australian *Banksia* woodland, recruitment from the seed bank was found to be profoundly affected by the season in which the *in situ* smoke treatment was applied (ROCHE, DIXON and PATE, 1998). Application of the smoke to unburnt sites in autumn promoted a significantly greater germination response than treatment in winter or spring for the majority of the species investigated. Interestingly, the promotive effect also extended beyond the initial year of application.

Several other studies have also been conducted using smoke treatments on soil seed bank samples (e.g. READ, BELLAIRS, MULLIGAN and LAMB, 2000; ENRIGHT and KINTRUP, 2001; WILLS and READ, 2002; TANG, BOULTER and KITCHING, 2003). These type of studies provide essential information for forest restoration, conservation, management and weed control, and illustrate the importance of investigating other fire-related cues along with smoke-stimulated germination (e.g. heat, see **Section 2.2.8**).

2.6.6 Habitat restoration and conservation

In the Western Cape Province of South Africa, rehabilitation programmes are taking place to remove dense stands of invasive alien trees and shrubs that threaten fynbos vegetation and water resources (RICHARDSON, MACDONALD, HOFFMANN and HENDERSON, 1997; VAN WILGEN, LE MAITRE and COWLING,1998). HOLMES, RICHARDSON, VAN WILGEN and GELDERBLOM (2000) have studied the recovery of South African fynbos following alien woody plant clearing and fire. It is important that the indigenous vegetation recovers quickly. If not, the soils are susceptible to erosion and the further colonization by fynbos species is slow. An important strategy for

restoring guild structure is to sow locally harvested indigenous seed in late summer or autumn, thereby augmenting the restoration of fynbos vegetation in restoration areas (HOLMES and RICHARDSON, 1999). Although many of the sites are burned prior to restoration, it may be possible to use smoke technology to enhance the germination of many of the indigenous species. Another important consideration is that smoke technology can be used for habitat restoration in urban areas where fires are undesirable (VAN STADEN, BROWN, JÄGER and JOHNSON, 2000).

In the United Kingdom, programmes are underway to restore large areas of heathland. Rehabilitation techniques involve the sowing of heather (*Calluna vulgaris*), the seeds of which can be strongly dormant. In an attempt to improve germination of this species, THOMAS and DAVIES (2002) tested various dormancy-breaking treatments, including smoke solutions. It was found that a combined treatment with smoke solution and $GA_{4/7}$ was more effective than smoke alone in promoting germination, and demonstrated the potential of these methods for improving germination of this species for large-scale rehabilitation.

There is a great potential for the use of smoke in assisting the revegetation and restoration of mined areas. For example, ROCHE, KOCH and DIXON (1997) used smoke techniques for the rehabilitation of bauxite mines in the jarrah forests in Western Australia. They found a 48-fold increase in total number of germinants and a 4-fold increase in species richness when aerosol smoke was applied to undisturbed jarrah forest. ROCHE, KOCH and DIXON (1997) also investigated the relative effectiveness of different smoke application methods for the improved germination of ten target species of interest for bauxite mine rehabilitation. Results showed that, in general, the use of aerosol smoke was more effective at promoting germination than pre-imbibition or smoke water application. However, germination for all the smoke treatments was significantly higher than the controls.

Although it is an important tool, the effectiveness of using smoke for revegetation will depend on the species composition of the topsoil seed bank (READ, BELLAIRS, MULLIGAN and LAMB, 2000). Smoke treatments may be applied prior to sowing, or storage, as described earlier. A study by ENRIGHT, GOLDBLUM, ATA and ASHTON

(1997) on *Eucalyptus* woodlands of Victoria has suggested that species which are not smoke-responsive are not adversely affected by smoke treatments. These findings support the use of smoke as a routine treatment for broadcast seed, a technique which is utilised by some Australian mining companies to improve the seedling recruitment and biodiversity within rehabilitated areas (ROCHE, KOCH and DIXON,1997; GRANT and KOCH, 1997; WARD, KOCH and GRANT, 1997; LLOYD, BARNETT, DOHERTY, JEFFREE, JOHN, MAJER, OSBORNE and NICHOLS, 2002).

A recent report by ROKICH, DIXON, SIVASITHAMPARAM and MENEY (2002), following a study of woodland restoration in sand quarries in Western Australia, suggest that the application of smoke water sprayed over the soil surface is an effective means for broad-scale restoration. However, this method would require large quantities of smoke water and is not always very practical, although a preferable method to using aerosol smoke. LLOYD, DIXON and SIVASITHAMPARAM (2000) have investigated the use of concentrated smoke products for the potential use of rehabilitating degraded landscapes. Results from the study suggest that concentrated smoke products could be economical and beneficial for broad-scale revegetation programmes.

Smoke treatments can also be used to improve the germination success of many rare and threatened species, such as *Tetratheca juncea,* a vulnerable Australian species (BELLAIRS, BARTIER, GRAVINA and BAKER, 2006). Smoke solutions are now regularly used in studies investigating dormancy-breaking treatments. For example, in a study on four Australian native species which are difficult to germinate, smoke solutions were used in an attempt to find suitable treatments for promoting the germination of these local species for re-establisment at mine sites (ALLAN, ADKINS, PRESTON and BELLAIRS, 2004). Smoke, therefore, has the potential for use in sustainable maintenance of existing populations, as well as in the development of effective protocols for establishing new populations of threatened species (e.g. BROWN and BOTHA, 2004).

2.7 SUMMARY

- In regions where wildfires form an integral part of the ecology, it is well known that fire plays an important role in seed germination
- The stimulatory effect of fire may be as a result of physical effects of heat on the seed coat, physiological effects on seed embryos, or the release of dormancy breaking compounds
- DE LANGE and BOUCHER (1990) reported the first study showing that cold smoke could induce germination of *Audouinia capitata*
- Further studies on South African fynbos, Californian chaparral and Australian species have shown the widespread ability of smoke to stimulate germination
- Smoke enables germination in species previously thought difficult to germinate, although not all species respond to smoke treatments
- Smoke can promote earlier and more uniform germination
- The promotive effect of smoke is independent of seed size and improves germination of species with different plant life forms
- Seeds can be treated with smoke through direct aerosol smoking, or through transfer via water or solid media
- High concentrations of smoke can inhibit germination
- The germination cue is retained once seeds are exposed to smoke, a factor which allows for smoke to be used as a pre-treatment or primer
- Although the mechanism for smoke-stimulated seed germination is not yet known, studies with exogenously applied plant hormones indicate the smoke seems to increase the sensitivity of the seeds to plant hormones
- There is potential for the application of smoke technology in horticulture, agriculture, weed control, ecological studies, habitat rehabilitation and conservation

3 The Lettuce Seed Bioassay

Hope is to life what seeds are to the earth.

~ Dutch Sheets ~
Tell Your Heart to Beat Again

3.1 INTRODUCTION

Lettuce (*Lactuca sativa* L.) achenes (hereafter referred to as seeds) have been used as a model for investigating various aspects of seed germination. The scientific literature contains numerous studies on lettuce seeds, many dealing with aspects of light-mediated seed germination and thermoinhibition. **Table 3.1** lists various studies on **Grand Rapids lettuce**, a variety which has a light requirement for germination. Mature seeds of Grand Rapids lettuce do not germinate in the dark, at temperatures that are suitable for germination, and are termed "light-sensitive", "photosensitive", "photoblastic" or "skotodormant".

Table 3.1: Examples of studies on Grand Rapids lettuce seed germination.

Publication Title	Reference
A reversible photoreaction controlling seed germination	BORTHWICK, HENDRICKS, PARKER, TOOLE and TOOLE, 1952
Action of light on lettuce-seed	BORTHWICK, HENDRICKS, TOOLE and TOOLE, 1954
Effect of gibberellin on germination of lettuce seed	KAHN, GOSS and SMITH, 1957
Photosensitive site in lettuce seeds	IKUMA and THIMANN, 1959
Action of gibberellic acid on lettuce seed germination	IKUMA and THIMANN, 1960
An analysis of "dark-osmotic inhibition" of germination of lettuce seeds	KAHN, 1960
The role of the seed-coats in germination of photosensitive lettuce seeds	IKUMA and THIMANN, 1963
The effects of temperature on the germination and radicle growth of photosensitive lettuce seed	IKUMA, 1964
Heat inactivation of gibberellic acid-sensitivity of lettuce seed germination	HABER, 1965

Table 3.1 continued...

Publication Title	Reference
Lettuce seed germination: Evidence for a reversible light-induced increase in growth potential and for phytochrome mediation of the low temperature effect	SCHEIBE and LANG, 1965
The effect of temperature and light on the germination of lettuce seeds	BERRIE, 1966
Induction of light sensitive dormancy in seed of *Lactuca sativa* L. (lettuce) by patulin	BERRIE, HENDRIE, PARKER and KNIGHTS, 1967
Lettuce seed germination: A phytochrome-mediated increase in the growth rate of lettuce seed radicles	SCHEIBE and LANG, 1967
Immediate phytochrome action in lettuce seeds and its interaction with gibberellins and other germination promoters	BEWLEY, NEGBI and BLACK, 1968
Inhibition of gibberellic acid-induced germination by abscisic acid and reversal by cytokinins	KHAN, 1968
Stimulation of lettuce seed germination by ethylene	ABELES and LONSKI, 1969
Synergistic action of ethylene with gibberellin or red light in germinating lettuce seeds	BURDETT and VIDAVER, 1971
Seed water content in relation to phytochrome-mediated germination of lettuce seeds (*Lactuca sativa* L. var. Grand Rapids)	HSIAO and VIDAVER, 1971
A distinction between the actions of abscisic acid, gibberellic acid and cytokinins in light-sensitive lettuce seed	BEWLEY and FOUNTAIN, 1972
Actions of gibberellic acid and phytochrome on the germination of Grand Rapids lettuce seeds	VIDAVER and HSIAO, 1974
Endogenous abscisic acid levels in germinating and nongerminating lettuce seed	BRAUN and KHAN, 1975
Photomanipulation of phytochrome in lettuce seeds	KENDRICK and RUSSELL, 1975
Additive and synergistic effects of kinetin and ethrel on germination, thermodormancy, and polyribosome formation in lettuce seeds	RAO, SANKHLA and KHAN, 1975
Abscisic acid as an endogenous component in lettuce fruits, *Lactuca sativa* L. cv. Grand Rapids. Does it control thermodormancy?	BERRIE and ROBERTSON, 1976
Variation in germination and amino acid leakage of seeds with temperature related to membrane phase change	HENDRICKS and TAYLORSON, 1976
Promotive effects of organic solvents and kinetin on dark germination of lettuce seeds	RAO, BRAUN and KHAN, 1976
Mode of action of gibberellic acid and light on lettuce seed germination	LEWAK and KHAN, 1977
Light effects upon dry lettuce seeds	McARTHUR, 1978

Table 3.1 continued...

Publication Title	Reference
The influence of plant growth regulators on the growth of the embryonic axes of red- and far-red-treated lettuce seeds	CARPITA, ROSS and NABORS, 1979
Effect of temperature on the short chain fatty acid-induced inhibition of lettuce seed germination	STEWART and BERRIE, 1979
Changes in the strength of lettuce endosperm during germination	TAO and KHAN, 1979
Red-light- and gibberellic-acid-enhanced α-galactosidase activity in germinating lettuce seeds, cv. Grand Rapids	LEUNG and BEWLEY, 1981
The effect of growth retardants on phytochrome-induced lettuce seed germination	GARDNER, 1983
Long-term storage of dormant Grand Rapids lettuce seeds in the imbibed state: physiological and metabolic changes	POWELL, LEUNG and BEWLEY, 1983
Interactions between hydrogen cyanide, gibberellin, abscisic acid and red light in germination of lettuce seeds	ZAGÓRSKI and LEWAK, 1983
Photoperceptive site in phytochrome-mediated lettuce (*Lactuca sativa* L. cv. Grand Rapids) seed germination	INOUE and NAGASHIMA, 1991
Evidence for inhibitor involvement in thermodormancy of Grand Rapids lettuce seeds	SMALL and GUTTERMAN, 1991
Light effects on endogenous levels of gibberellins in photoblastic lettuce seeds	TOYOMASU, TSUJI, YAMANE, NAKAYAMA, YAMAGUCHI, MUROFUSHI, TAKAHASHI and INOUE, 1993
Effects of exogenously applied gibberellin and red light on the endogenous levels of abscisic acid in photoblastic lettuce seeds	TOYOMASU, YAMANE, MUROFUSHI and INOUE, 1994
Roles of soluble sugars in protecting phytochrome- and gibberellin A_3-mediated germination control in skotodormant seeds	HSIAO and QUICK, 1997
Phytochrome regulates gibberellin biosynthesis during germination of photoblastic lettuce seeds	TOYOMASU, KAWAIDE, MITSUHASHI, INOUE and KAMIYA, 1998
Restoration of seed germination at supraoptimal temperatures by fluridone, an inhibitor of abscisic acid biosynthesis	YOSHIOKA, ENDO and SATOH, 1998
Deactivation of gibberellin by 2-oxidation during germination of photoblastic lettuce seeds	NAKAMINAMI, SAWADA, SUZUKI, KENMOKU, KAWAIDE, MITSUHASHI, SASSA, INOUE, KAMIYA and TOYOMASU, 2003
A method for profiling classes of plant hormones and their metabolites using liquid chromatography-electrospray ionization tandem mass spectrometry: an analysis of hormone regulation of thermodormancy of lettuce (*Lactuca sativa* L.) seeds	CHIWOCHA, ABRAMS, AMBROSE, CUTLER, LOEWEN, ROSS and KERMODE, 2003

Table 3.1 continued...

Publication Title	Reference
The regulation of the thermoinhibition of seed germination in winter annual plants by abscisic acid	YOSHIOKA, GONAI, KAWAHARA, SATOH and HASHIBA, 2003
Abscisic acid in the thermoinhibition of lettuce seed germination and enhancement of its catabolism by gibberellin	GONAI, KAWAHARA, TOUGOU, SATOH, HASHIBA, HIRAI, KAWAIDE, KAMIYA and YOSHIOKA, 2004

The perception of light by seeds is controlled by **phytochrome**, an important family of photoreceptors in higher plants that are involved in many photomorphogenetic reactions. Phytochrome consists of a protein bound to a light-absorbing pigment (chromophore), which exists as two interconvertible isomers, resulting in the photoreversible properties of phytochrome (HENDRICKS and BORTHWICK, 1967; LEYSER and DAY, 2003). The red light absorbing form (Pr) absorbs light in the red part of the spectrum (peak absorbance at 660 nm), and the far-red light absorbing form (Pfr) absorbs light in the far-red region (peak absorbance at 730 nm). Generally, germination is initiated when phytochrome is in the Pfr form, and is either prevented or has no effect on germination when in the Pr form (PONS, 2000). Furthermore, brief pulses of red light promote germination, but can be reversed by immediate irradiation with far-red light (SCHEIBE and LANG, 1965). Recent advances in phytochrome research are discussed by CASAL and SÁNCHEZ (1998).

The complex process of germination also involves the synthesis, metabolism and sensitivity of tissues to **plant hormones**. In particular, gibberellins (GAs) and cytokinins (CKs) play an important role in seed germination, along with abscisic acid (ABA) which generally has an inhibitory role (THOMAS, 1992). From studies, such as those in **Table 3.1**, Grand Rapids lettuce seeds are well-characterized with respect to their response to red light and far-red light, GAs, ABA and ethylene. In particular, GAs are known to break the dormancy of Grand Rapids lettuce seeds (VIDAVER and HSIAO, 1974; LEWAK and KHAN, 1977).

DREWES, SMITH and VAN STADEN (1995) demonstrated that **smoke solutions** can overcome the light-requirement of Grand Rapids lettuce seeds. The promotion of germination by smoke solutions can be detected after 24 h, making the lettuce seed

bioassay a useful tool for the detection of germination-promoting compounds from plant-derived smoke. Furthermore, this simple and rapid bioassay provided a useful system for bioactivity-guided fractionation in the process of isolating a germination promoter from smoke (DREWES, SMITH and VAN STADEN, 1995; see **Chapter 6**). It was used extensively in the experiments presented in this thesis and is discussed below in more detail.

3.2 MATERIALS AND METHODS

3.2.1 Lettuce seed bioassay

The following procedure for the lettuce seed bioassay was used for testing germination activity in almost all the experiments presented in this thesis, unless otherwise indicated. Mature achenes of *Lactuca sativa* L. cv. Grand Rapids (Peto Seed, Saticoy, USA) were stored in the dark at 4 °C until use. All manipulations of seeds were performed in the dark with a green "safelight" (0.5 μmol m^{-2} s^{-1}) (DREWES, SMITH and VAN STADEN, 1995). Seeds were placed in 65 mm plastic Petri dishes lined with two layers of Whatman No. 1 filter paper moistened with 2 mL of test solution (e.g. smoke solution, fraction sample). Distilled water was used as a control. The seeds were kept in a light-proof box and incubated at 25 ± 0.5 °C for 24 h. In all experiments, four replicates of 25 seeds per treatment were used, unless otherwise stated. Seeds were considered to have germinated when the radicle had visibly (to the naked eye) emerged through the seed coat.

A smoke extract produced in 1994 from burnt *Themeda triandra*, according to the method outlined by BAXTER, VAN STADEN, GRANGER and BROWN (1994), was used at a dilution of 1:1000 (pH 4.7) as a standard solution (positive control). This has been established as the concentration giving optimal germination of Grand Rapids lettuce seeds (DREWES, SMITH and VAN STADEN, 1995). **Figure 3.1** shows the apparatus used for producing smoke-saturated water (also see **Section 2.3.2.2**). Smoke from smouldering *T. triandra* leaf material was bubbled through 500 mL of distilled water for 45 min. The extract was stored at 10 °C with no change in germination

activity observed over the storage period (1994 to present).

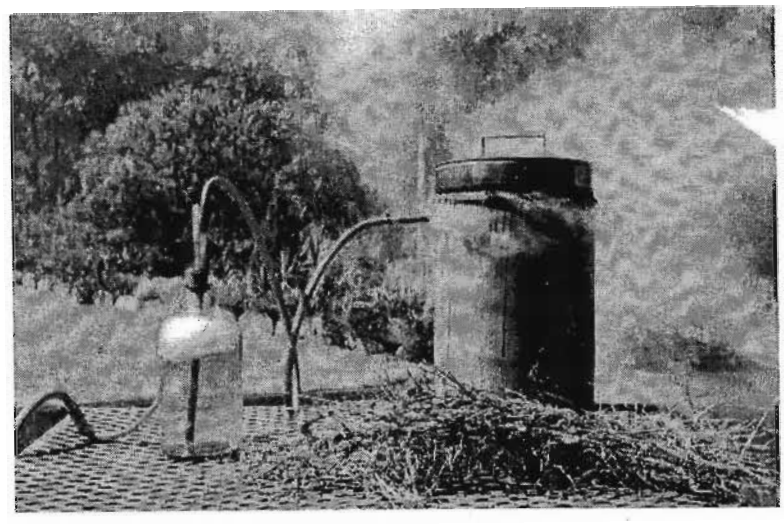

Figure 3.1: Apparatus for producing aqueous smoke extracts.

3.2.2 Statistical analysis

Germination data were arcsine transformed and analysed using a one-way analysis of variance (ANOVA) and a Tukey's multiple range test, at a 5% level of significance (SOKAL and ROHLF, 1987). MINITAB™ release 13.1 was used to conduct the analyses.

3.3 RESULTS AND DISCUSSION

3.3.1 Response to smoke treatments

Grand Rapids lettuce seeds imbibed in a smoke solution showed no differences in the **rate of imbibition** in comparison to the control (LIGHT, GARDNER, JÄGER and VAN STADEN, 2002). **Figure 3.2** illustrates the imbibition curves, over a period of 6 h, for seeds treated with water or with a 1:1000 dilution of smoke solution. Approximately 30 min after the start of imbibition the seeds were 25% hydrated, and are fully imibibed within 6 h.

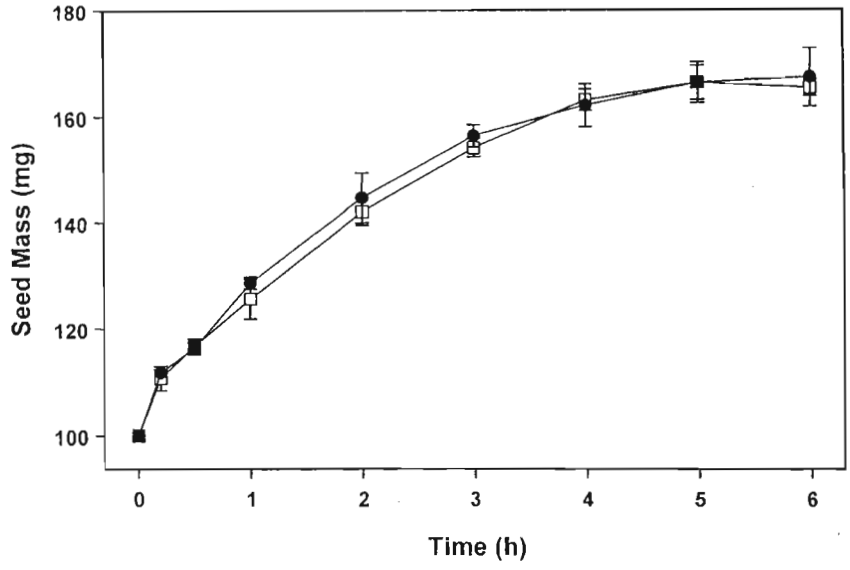

Figure 3.2: Imbibition curves for Grand Rapids lettuce seeds imbibed in (●) water, or (□) 1:1000 dilution of smoke extract. Error bars indicate SD. (From LIGHT, GARDNER, JÄGER and VAN STADEN, 2002).

The treatment of Grand Rapids lettuce seeds with a smoke solution, or active samples after fractionation, produced a rapid germination response after 24 h in the dark at 25 ˚C (**Figure 3.3**).

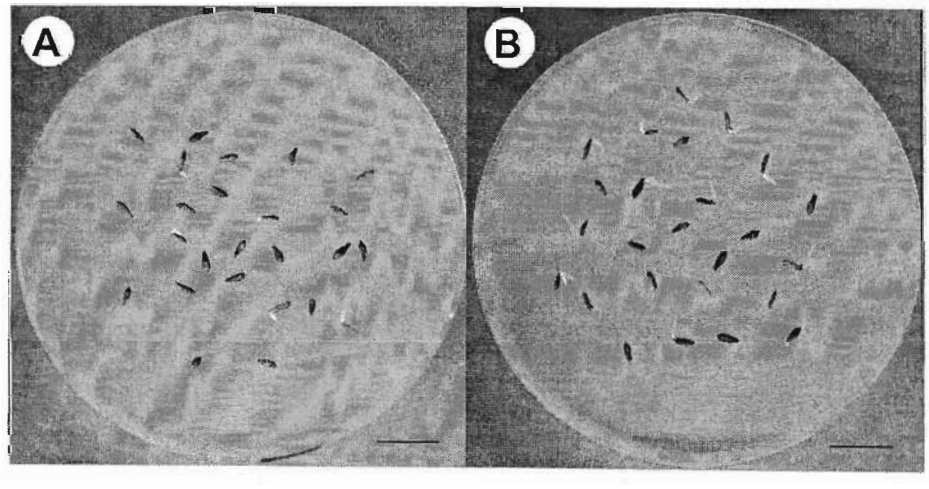

Figure 3.3: Typical germination response obtained with Grand Rapids lettuce seeds after 24 h in the dark at 25 ˚C treated with (A) distilled water, or (B) 1:1000 dilution of smoke extract or active fraction.

Treating seeds with different dilutions of the *Themeda triandra* smoke solution, resulted in a response curve not unlike phytohormone response curves (**Figure 3.4**; DREWES, SMITH and VAN STADEN, 1995). Undiluted smoke extract and a 1:10 dilution totally inhibited germination, whereas a dilution of 1:100 gave moderate levels of germination. However, lower dilutions show significantly increased germination compared to water controls, with the 1:1000 dilution giving the best result. Hence, this dilution of smoke solution was used as a "standard" in the bioassay when testing fractions of purified smoke extracts.

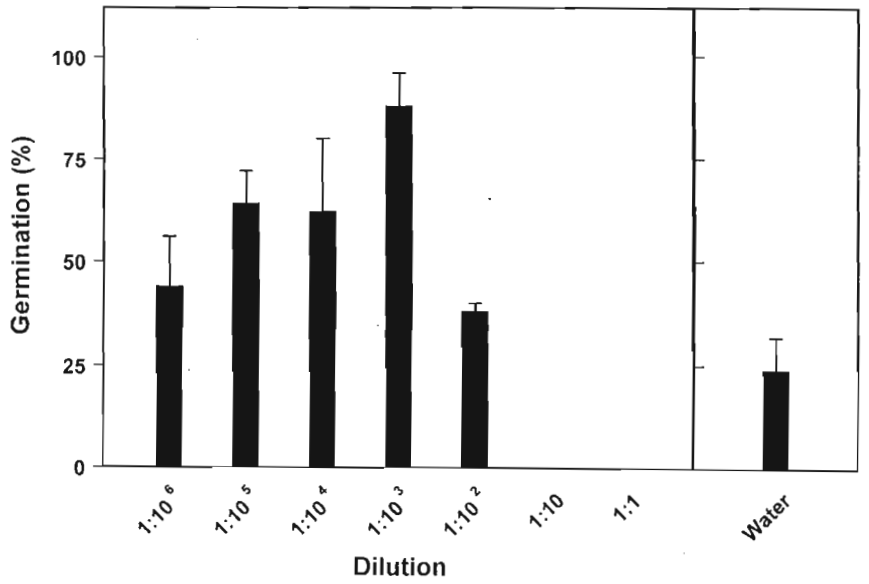

Figure 3.4: Effect of different dilutions of smoke extract on the germination of Grand Rapids lettuce seeds after 24 h in the dark at 25 °C. Error bars indicate SE.

It is important to note, however, that seeds from different sources or batches do not respond equally. For example, VAN STADEN, DREWES and BROWN (1995) tested six seed lots of Grand Rapids lettuce from different sources or batches. Treatments with red light and far-red light illustrated the differing degrees of sensitivity to light treatments, as well as smoke treatments. Thus, for use in a bioassay, it is important to use a seed lot that provides suitable levels of germination, both for the dark control and in response to smoke solutions. In seed lots where the control germination is relatively high (i.e. a high level of Pfr), a 10 min exposure to far-red light, one hour after the start of imbibition in the dark, can depress germination in order to make the smoke response more obvious.

For the preparation of smoke products, it is important to check that smoke solutions provide a suitable germination response (see **Section 2.3.3.2**). Light-sensitive lettuce seeds thus provide an ideal test system, although the sensitivity and response of the seed lot should be known and less-sensitive seeds should not be used. BOUCHER and MEETS (2004) used Grand Rapids lettuce seed to evaluate the relative activity of five types of smoke solutions. Their study did show some small differences in germination responses for the solutions tested. However, in comparison to the high germination levels obtained with the lettuce seeds used in our studies (usually > 80%, see **Figure 3.4**), they obtained maximum germination levels of approximately 40% with smoke treatment and lower levels for the water control. It is possible that the seeds used in their study were not highly smoke-sensitive. Furthermore, it is also possible that fungicide treatments applied by seed companies may interfere with the uptake of stimulatory substances in smoke solutions, thus making those seeds unsuitable for use in a bioassay system. It may also be possible to use other varieties of lettuce for use in testing smoke solutions, although not all lettuce varieties have light-sensitive seeds. For examples, the lettuce variety "Ritsa" has light-sensitive seeds, whereas the variety "Strada" is light-insenstive (ROTH-BEJERANO, SEDEE, VAN DER MEULEN and WANG, 1999).

Lettuce seeds are not the only seeds that have been used as a bioassay species for investigating smoke-stimulated seed germination. For example, BALDWIN, STASZAK-KOZINSKI and DAVIDSON (1994) used seeds of *Nicotiana attenuata* to test fractions of smoke extracts and pure chemicals for their ability to influence germination. In this species, maximum germination was obtained after 7 days. ROCHE, KOCH and DIXON (1997) used *Lysinema ciliatum* (curry and rice) as a bioassay species, to test smoke solutions, and TIEU, DIXON, SIVASITHAMPARAM, PLUMMER and SIELER (1999) used *Stylidium affine* to ensure that the smoke solution used in their study was comparable in activity to smoke solutions used in other studies.

3.3.2 Effect of red light and far-red light on smoke-treated seeds

Grand Rapids lettuce seeds respond, as would be expected, to classic red/far-red light

treatments (DREWES, SMITH and VAN STADEN, 1995). In the dark, at temperatures suitable for germination, very low levels of germination are observed. A red light treatment, when applied as the last treatment prior to incubation in the dark, stimulates germination, whereas a far-red light treatment inhibits germination. When applied immediately following each other, these treatments (red/far-red light) are reversible. Thus, the control of germination in these seeds is strongly dependent on the phytochrome system.

JÄGER and VAN STADEN (2002) found that Grand Rapids lettuce seeds achieved maximum germination when the red light treatment was given 5 min and onwards after the start of imbibition. Within the first two hours of imbibition, the red light treatment could be completely reversed with a 30 min treatment of far-red light. Thereafter, the effect of the far-red light diminished, with a maximum escape time of 5 h (JÄGER and VAN STADEN, 2002).

DREWES, SMITH and VAN STADEN (1995) found that exposing smoke-treated Grand Rapids lettuce seeds to 20 min or more of far-red light overcame the stimulatory effect of the smoke extract. Thus, it appears that smoke either substitutes for red light and affects the conversion of Pr to Pfr, or is involved in some way in the phytochrome response. However, a further study on the interaction between smoke and different plant hormones indicated that smoke may affect membrane permeability or receptor sensitivity rather than influencing the phytochrome system (see below; VAN STADEN, JÄGER and STRYDOM, 1995).

A time course analysis (2 h intervals) of red light and smoke-treated Grand Rapids lettuce seeds showed that red light treatments induced the most rapid germination response (GARDNER, DALLING, LIGHT, JÄGER and VAN STADEN, 2001). For the red light treatment, germinating seeds were visible from 8 h onwards, with 98% germination obtained after 12 h. Smoke-treated seeds, however, showed a delayed response, with germination beginning at 12 h and steadily increasing to a maximum of 69% after 24 h.

3.3.3 Interaction of plant hormones with smoke

Several studies on the interaction of plant hormones and smoke-stimulated germination of Grand Rapids lettuce seeds have been conducted. A summary of these studies is presented in **Table 3.2** and discussed further. From these studies, smoke solutions have been shown to interact with GAs, CKs, ethylene and ABA, sometimes increasing the sensitivity of the seeds to exogenously applied hormones (VAN STADEN, BROWN, JÄGER and JOHNSON, 2000). In terms of hormonal control, phytochrome-mediated dormancy is a complex phenomenon. There is some evidence that various plant hormones, particularly GAs, CKs, ethylene and ABA are involved in, or associated with, the phytochrome control system of photoblastic seeds (THOMAS, 1992).

Table 3.2: Summary of studies investigating plant hormones and smoke-stimulated germination of Grand Rapids lettuce seeds. (ABA = abscisic acid; BA = N^6-benzyladenine; FR = far-red light; GA_3 = gibberellic acid; R = red light; SM = smoke solution).

Publication title/Reference	Main findings
Interaction between a plant-derived smoke extract, light and phytohormones on the germination of light-sensitive lettuce seeds VAN STADEN, JÄGER and STRYDOM, 1995	• A similar effect with SM or GA_3 (10^{-4} M) with different R/FR treatments • BA or zeatin alone did not promote germination; a small synergistic effect of SM and BA • Ethephon (ethylene releasing compound) did not promote germination • Additive effect with GA_3 (10^{-4} M) and SM • At suboptimal SM concentrations, a synergistic effect between SM and GA_3 • Paclobutrazol (10^{-2} M) reduced the germination of SM-treated seeds, but not R-treated seeds • At 10^{-5} M ABA, SM could not overcome inhibition by ABA, whereas red light partially did • Combination of SM, ABA and red light resulted in lower germination than with ABA and red light • SM increased the sensitivity of seeds to ABA
Effect of a plant-derived smoke extract, N^6-benzyl-adenine and gibberellic acid on the thermodormancy of lettuce seeds STRYDOM, JÄGER and VAN STADEN, 1996	• Seeds with induced thermodormancy, treated with SM, BA or GA_3 alone did not germinate in the light at 25 °C • BA (10^{-3} to 10^{-5} M) with SM dilutions overcame thermodormancy • BA (10^{-5} M) and GA_3 (10^{-4} M) overcame thermodormancy • GA_3 and SM had no effect (unless with BA)

Table 3.2 continued...

Publication title/Reference	Main findings
The effect of ethylene, octanoic acid and a plant-derived smoke extract on the germination of light-sensitive lettuce seeds JÄGER, STRYDOM and VAN STADEN, 1996	• Octanoic acid did not promote germination • Ethylene, at 5 to 100 µL L^{-1} increased germination (not as much as optimal SM dilution) • At high SM concentration, effect of ethylene inhibited • At suboptimal SM concentrations, synergistic effect with ethylene • 1 mM octanoic acid with ethylene, greater germination than with ethylene alone • The active compound in smoke is unlikely to be octanoic acid or ethylene
Does smoke substitute for red light in the germination of light-sensitive lettuce seeds by affecting gibberellin metabolism? GARDNER, DALLING, LIGHT, JÄGER and VAN STADEN, 2001	• Germination of SM-treated seeds delayed compared to seeds treated with R or GA_3 • R and SM can be reversed by FR, but escape time shorter for SM • Paclobutrazol and AMO 1618 (GA biosynthesis inhibitors) reduced germination of seeds treated with SM, but not R • Endogenous GA levels higher in R-treated seeds • GA levels in SM seeds rose with start of germination • GA synthesis considered to be component of mechanism for SM-induced germination
Soluble sugars in light-sensitive Grand Rapids lettuce seeds treated with red light, gibberellic acid and a plant-derived smoke extract JÄGER and VAN STADEN, 2002	• The escape time for FR reversal (30 min) was found to be 5 h • An increase in sucrose 2 h after start of imbibition, in all treatments (SM, GA_3, R), including control • Induction of germination not totally dependent on utilisation of soluble sugars

Gibberellins (GAs) are known to promote the germination of light-sensitive lettuce seeds in the dark, and a close relationship between the action of Pfr and GA has been demonstrated (VIDAVER and HSIAO, 1974). Additionally, evidence from studies on *Arabidopsis* germination has shown that phytochromes mediate their effect on germination by influencing GA biosynthesis and sensitivity (LEYSER and DAY, 2003). In general, GAs are primarily involved in metabolic processes leading to embryo growth and radicle emergence, such as energy reserve mobilisation and endosperm softening (THOMAS, 1992).

For Grand Rapids lettuce, VAN STADEN, JÄGER and STRYDOM (1995) found that similar germination levels were observed for seeds treated with smoke solutions or GA_3

(10^{-4} M) in response to different red light and far-red light treatments, and in the dark. A synergistic effect of GA_3 in combination with smoke was observed, and of all the hormones tested in the study, GA_3 showed the greatest degree of interaction with smoke in the germination of Grand Rapids lettuce seeds.

Paclobutrazol is known to inhibit the oxidation of *ent*-kaurene to *ent*-kaurenoic acid, an early step in GA biosynthesis. Treatment of Grand Rapids lettuce seeds with 10^{-2} M paclobutrazol reduced the germination of seeds treated with 10^{-4} M GA_3 and smoke solution. Seeds treated with red light, however, were not inhibited (VAN STADEN, JÄGER and STRYDOM, 1995). An inhibition of red light stimulated germination, by GA biosynthesis inhibitors, can only be expected if phytochrome initiates a *de novo* synthesis of GAs, and is not involved in activating a pool of inactive forms of GA (VAN STADEN, JÄGER and STRYDOM, 1995). In a further study, smoke-stimulated germination of Grand Rapids lettuce seeds was shown to be reduced by paclobutrazol at 10^{-4} and 10^{-3} M. Germination of red light treated seeds, however, was only slightly reduced by this inhibitor. Treatment of seeds with smoke solution and AMO 1618, which interferes with geranylgeranyl pyrophosphate conversion in GA biosynthesis, showed a similar trend, but to a much lesser extent. Germination of seeds treated with exogenous application of GA_3 was not significantly reduced with the addition of paclobutrazol or AMO 1618 (GARDNER, DALLING, LIGHT, JÄGER and VAN STADEN, 2001).

GARDNER, DALLING, LIGHT, JÄGER and VAN STADEN (2001) measured levels of putative endogenous GAs, using the dwarf rice microdrop assay, in red light- and smoke-treated Grand Rapids lettuce seeds. In the red light treated seeds, a rapid increase in GA was observed, reaching a maximum of 0.9 ng g^{-1} after 14 h, 2 h after maximum germination had been reached. In the smoke-treated seeds, a maximum GA level of 7.4 ng g^{-1} was found after 18 h, before maximum germination was attained. Thus, is seems likely that GA synthesis is a likely component of the mechanism involved in smoke-stimulated germination, and may act indirectly via modulation of the endosperm structure (GARDNER, DALLING, LIGHT, JÄGER and VAN STADEN, 2001).

It is generally considered that **abscisic acid (ABA)** is involved in the establishment and

maintenance of primary dormancy. In tests with non-dormant seeds, ABA counteracts the effects of GAs, CKs and ethylene. Various studies have shown that GAs and ABA act independently, whereas ABA effects can sometime be reversed by high concentrations of CKs. However, GA_3 is known to overcome the inhibitory effects of ABA on the germination of lettuce seeds (THOMAS, 1992). VAN STADEN, JÄGER and STRYDOM (1995) found that Grand Rapids lettuce seeds treated with 10^{-5} M ABA and smoke, and irradiated with red light, showed a greater reduction in germination than seeds treated with ABA and red light only. Thus, it seems that smoke somehow increases the sensitivity of the lettuce seeds to ABA.

Cytokinins (CKs) are generally only able to induce germination in light-sensitive seeds in the presence of GAs or with pulses of red light (THOMAS, 1992). In Grand Rapids lettuce seeds, the application of BA or zeatin did not increase germination when applied on their own, and only a minor synergistic effect was observed with BA in combination with smoke (VAN STADEN, JÄGER and STRYDOM, 1995). Grand Rapids lettuce seeds enter a state of thermodormancy when exposed to high temperatures (30 to 38 °C) for extended periods. STRYDOM, JÄGER and VAN STADEN (1996) showed that for thermodormant lettuce seeds, dormancy could be partially broken with BA at 10^{-5} M. In combination with a smoke solution BA was more effective, and also showed higher levels of germination than the GA_3 plus smoke treatment.

It is well known that **ethylene** promotes germination in seeds from a wide range of species. However, it does not appear to have an effect on the germination of Grand Rapids lettuce seeds in the absence of light (ABELES and LONSKI, 1969). VAN STADEN, JÄGER and STRYDOM (1995) observed that ethephon, an ethylene releasing compound, did not promote germination of Grand Rapids lettuce seeds in the dark. A further study, however, showed that ethylene, at concentrations from 5 to 100 $\mu L\ L^{-1}$ did increase germination of Grand Rapids lettuce seeds (JÄGER, STRYDOM and VAN STADEN, 1996). However, this increase was not to the level observed with optimal dilutions of a smoke solution. A synergistic effect was, however, observed with suboptimal concentrations of smoke solution in combination with 5 $\mu L\ L^{-1}$ of ethylene (JÄGER, STRYDOM and VAN STADEN, 1996).

From these studies on the interaction of plant hormones and smoke solutions, it is still unclear how smoke acts to stimulate germination of light-sensitive lettuce seeds in the dark. The presence of compounds which inhibit germination at high concentrations of smoke solutions may also interfere with the effects observed in these studies. Hence, as discussed in **Section 2.5**, the isolation of the active germination promoter will now allow for more detailed studies on the physiology of seed germination, without the confounding effects of other chemicals present in smoke solutions (see **Chapter 6**; FLEMATTI, GHISALBERTI, DIXON and TRENGOVE, 2004; VAN STADEN, JÄGER, LIGHT and BURGER, 2004).

3.4 SUMMARY

- In the light, at 25 ˚C, seeds of *Lactuca sativa* L. cv. Grand Rapids germinate optimally within 24 h
- In the dark, at 25 ˚C, very low levels of germination are observed after 24 h
- In the dark, at 25 ˚C, high levels of germination are observed with smoke solutions (or purified samples of smoke solutions) after 24 h
- The lettuce seed bioassay therefore provides an ideal system for rapid activity testing of germination promoter(s) found in plant-derived smoke

4 Dual Nature of the Regulation of Germination by Smoke

God could cause us considerable embarrassment by revealing all the secrets of nature to us: we should not know what to do for sheer apathy and boredom.

~ Johann Wolfgang von Goethe (1749 - 1832) ~

4.1 INTRODUCTION

The observation by BALDWIN, STASZAK-KOZINSKI and DAVIDSON (1994) that the stimulatory effect of smoke is irreversible, is an important property of smoke-stimulated seed germination. They found that, once seeds of *Nicotiana attenuata* were soaked in a smoke-containing extract for more than 15 min, it was not possible to prevent germination by rinsing the seeds. This phenomenon has allowed smoke and smoke solutions to be utilized as effective seed pre-treatments (see **Section 2.6.3**; BAXTER and VAN STADEN, 1994; BROWN, PROSCH and BOTHA, 1998; BROWN and VAN STADEN, 1998).

Contrary to the retention of the germination cue, the inhibitory effects of moderately high concentrations of smoke solutions appear to be reversible (BROWN, 1993a; also see **Section 2.6.3**). In a study on the effect of smoke water on fynbos species, it was noted that seeds of *Syncarpha vestita* treated with a 1:2 concentration of smoke extract did not germinate. However, after being flushed with distilled water, the inhibitory effect of the high smoke concentration was relieved (BROWN, 1993a).

In order to gain further insight into the effect of smoke-stimulated germination, light sensitive Grand Rapids lettuce seeds were pulse-treated with smoke solutions. The storage of smoke-treated lettuce seeds, and the effect of higher smoke concentrations were also examined.

4.2 MATERIALS AND METHODS

4.2.1 Smoke pulse treatments

The duration of the minimum exposure time to smoke solutions necessary for the optimal germination of Grand Rapids lettuce seeds was investigated (**Section 3.2.1** describes the lettuce seeds and smoke extract used). This was determined by treating five replicates of 25 seeds placed in 65 mm plastic Petri dish fitted with two sheets of Whatman No. 1 filter paper moistened with 2 mL of a 1:1000 dilution of a smoke extract. Seeds were exposed to the smoke solution for 1, 15 and 30 min, and 1 hourly intervals up to 6 h and were then rinsed thoroughly in 25 mL distilled water prior to transferral to new Petri dishes containing 2 mL distilled water. Control treatments included seeds incubated in 2 mL distilled water or 2 mL of 1:1000 smoke solution for the duration of the germination trial. All manipulations were carried out in the dark under a green "safelight", and seeds were incubated in the dark at 25 °C. After 24 h, the number of germinated seeds were recorded (taken from the start of the application of smoke solution). Similarly, experiments were also carried out using 1:100 and 1:10 dilutions of the smoke extract.

4.2.2 Storage treatments

Germination trials were conducted to determine if there was a detrimental effect of treating seeds with high concentrations of smoke extract (dilution of 1:100) for long durations, and to see if pulse-treated seeds could retain the germination cue. As in the above experiments, five replicates of 25 seeds were used. Appropriate control treatments included 2 mL distilled water or 1:1000 and 1:100 dilutions of a smoke extract. All manipulations were carried out in the dark under a green "safelight", and seeds were incubated in the dark at 25 °C.

The various treatments were as follows:

(1) Seeds were pulse-treated for 1 h with 2 mL 1:100 smoke solution, thoroughly rinsed with 25 mL distilled water and transferred to new Petri dishes containing

2 mL water. The seeds were incubated in the dark at 25 °C for 24 h and germination was recorded.

(2) Seeds were treated with 2 mL 1:100 smoke solution and incubated in the dark at 25 °C. After 24 h the seeds were thoroughly rinsed with 25 mL distilled water and transferred to new Petri dishes containing 2 mL water. The seeds were then incubated for a further 24 h after which germination was recorded.

(3) The seeds were pulse-treated as in treatment 1. The seeds were thoroughly rinsed, blotted dry, and then dried in a stream of air. Seeds were stored in the dark at 4 °C. After a 14 day storage period, the seeds were moistened with 2 mL distilled water and incubated in the dark at 25 °C for 24 h and germination was recorded.

(4) This treatment followed the same procedure as treatment 3. However, seeds were rinsed after storage at 4 °C for 14 days (not before storage).

4.3 RESULTS AND DISCUSSION

4.3.1 Smoke pulse treatments

Exposure of the lettuce seeds to 1:1000 smoke solutions for periods of 1 h or less resulted in germination levels which did not differ significantly from the water control (**Figure 4.1A**). The percentage germination obtained for seeds exposed to smoke solutions for 2 h or longer did, however, display a marked increase above both the shorter-duration treatments and the water control, and there was a general linear increase in the percentage germination with time beyond the 2 h exposure period, up to 6 h. A maximum of 90% germination was attained for the 6 h pulse treatment (**Figure 4.1A**). Seeds treated with smoke solution for 5 or 6 h showed slightly higher levels of germination than the smoke controls (seeds exposed to smoke solution for 24 h).

Increasing the concentration of the smoke extract to 1:100 dilution resulted in an observable increase in the percentage germination obtained for shorter duration pulses (**Figure 4.1B**). Maximum germination was attained after a 1 h pulse and remained at this level for the longer duration pulses (**Figure 4.1B**). Levels of germination obtained

for 15 and 30 min pulses were noticeably higher than those observed for the 1:1000 dilution pulse treatments (**Figure 4.1A**). The elevated levels of germination after short duration pulses was also reflected in results obtained for 1:10 dilutions of the smoke extract (results not shown). Seeds which were pulse-treated with a 1:100 dilution of smoke extract for periods ranging from 15 min to 6 h showed a significantly higher germination response than seeds which were continuously exposed to the 1:100 smoke solution for 24 h (**Figure 4.1B**).

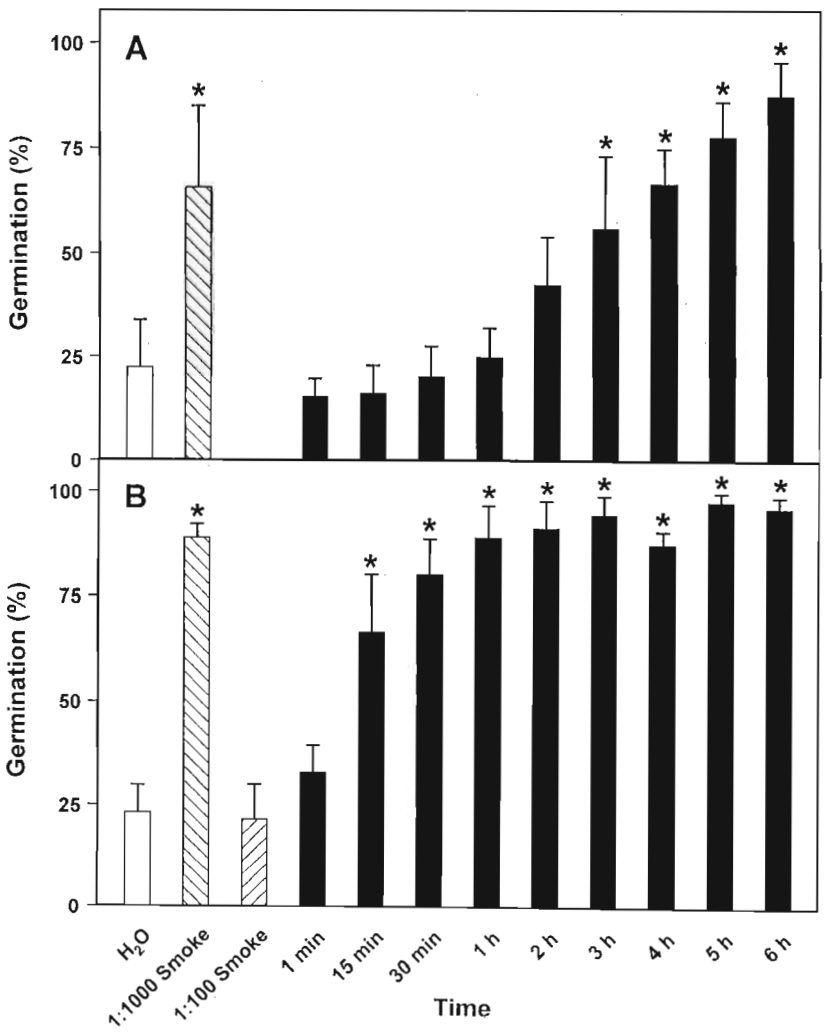

Figure 4.1: Germination of Grand Rapids lettuce seeds pulse-treated for different durations with (A) 1:1000, or (B) 1:100 dilutions of smoke extract after 24 h in the dark at 25 °C. Asterisks denote a significant ($p < 0.05$) difference from the water control; error bars indicate SD.

Results from these pulse treatments clearly indicate that exposure to the smoke cue for a minimum period is a conditional requirement for smoke-induced germination of lettuce seeds (**Figure 4.1A**). This implies that there is a requirement for a threshold level of the active smoke component in the seed. The reduction of the required exposure time by elevated concentrations of the smoke solution confirms this speculation (**Figure 4.1B**). Moreover, pulse treatments of 1:1000 smoke dilution resulted in a roughly linear increase in the percentage germination for periods exceeding 2 h (**Figure 4.1A**) This is consistent with the notion that the operative chemical in the smoke solution is responsible for the activation of an endogenous signalling mechanism. It also argues against a purely physical change in seed or membrane structure as the main causative mechanism in smoke-induced germination of lettuce seeds, as suggested by EGERTON-WARBURTON (1998).

Seeds which were continuously exposed to the 1:100 dilution of smoke extract for 24 h showed low levels of gemination compared to the pulse-treated seeds which showed a significant increase in germination (**Figure 4.1B**). While previous investigations have shown that prolonged exposure of seeds to high concentrations of smoke inhibit germination (DREWES, SMITH and VAN STADEN, 1995), these results indicate that a short pulse treatment of a high smoke concentration can elicit a high germination response. Hence, the detrimental effects of high smoke concentrations can be overcome by removal of the inhibitory compounds from the smoke. It is important to note that a similar treatment with a 1:10 smoke solution did, however, irreversibly damage the seeds, as evidenced by abnormal cotyledon expansion and seed coat rupture (results not shown).

4.3.2 Storage treatments

As was seen in the smoke pulse treatments, exposure of lettuce seeds to a 1:100 dilution of the smoke extract for 1 h, followed by incubation with 2 mL distilled water gave a significantly improved germination level (**Figure 4.2, Treatment 1**). However, continued exposure to the same concentration of smoke dilution for 24 h resulted in very low levels of germination (**Figure 4.2, 1:100 Smoke**). In comparison, seeds which were

exposed to a 1:100 smoke solution for 24 h showed a dramatically different response when rinsed with distilled water and incubated for a further 24 h (**Figure 4.2, Treatment 2**). Seeds which were pulse-treated with a 1:100 dilution of smoke extract for 1 h and stored for 14 days, with rinsing either prior to storage or after storage, showed similar levels of germination to seeds which were allowed to germinate immediately following an equivalent pulse treatment (**Figure 4.2, Treatment 1, 3 and 4**). Thus, the seeds retained the ability to germinate in the dark, even after a 2 week storage period.

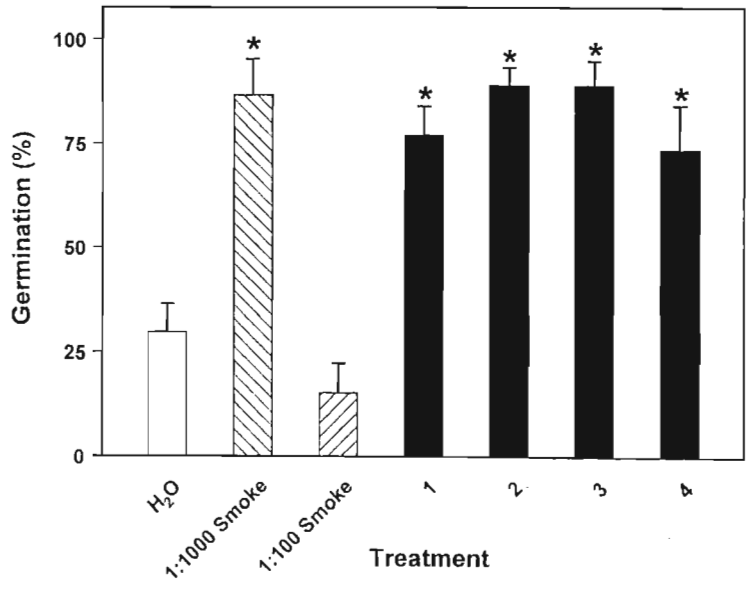

Figure 4.2: Germination of Grand Rapids lettuce seeds exposed to various treatments with 1:100 dilution of smoke extract. (1) 1 h pulse-treatment of smoke; (2) 24 h treatment of smoke, followed by rinsing; (3) 1 h pulse-treatment, rinsed, dried and stored for 14 days prior to germination; (4) 1 h pulse-treatment, dried and stored for 14 days, prior to rinsing and germination. Asterisks denote a significant ($p < 0.05$) difference from the water control; error bars indicate SD.

4.3.3 Dual nature of germination regulation

It is interesting to note that imbibition of lettuce seeds with water, prior to incubation with diluted smoke extract, resulted in reduced levels of germination in comparison to seeds imbibed continuously on smoke extract (**Figure 4.3**; LIGHT, GARDNER, JÄGER and VAN STADEN, 2002). This reduction of germination by pre-treatment with water implies that either the active component in smoke is required at a specific threshold level, or

that the activation of a barrier to solute movement, represented in the lettuce seed system by the endosperm layer, at 20-25% imbibition, accounts for the exclusion of the active component (OBROUCHEVA and ANTIPOVA, 1997). Therefore, the promotion of germination by smoke is dependent on the initial uptake of the active component, and most likely, on the early presence of this component in the embryonic axis of the seed (LIGHT, GARDNER, JÄGER and VAN STADEN, 2002).

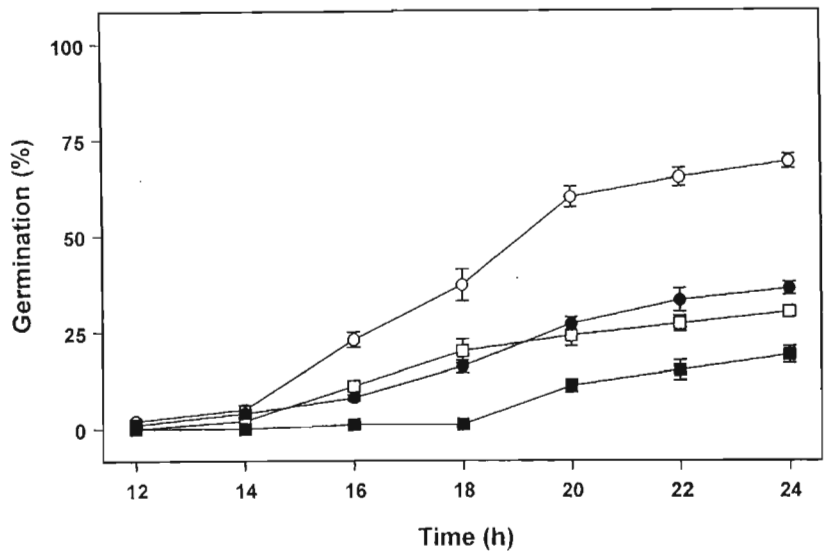

Figure 4.3: Germination of Grand Rapids lettuce seeds imbibed in water for (○) 0, (■) ½, (□) 1 or (●) 2 h prior to treatment with 1:1000 dilution of smoke extract. Error bars indicate SD. (From LIGHT, GARDNER, JÄGER and VAN STADEN, 2002).

The results from the experiments using pulse-treatments seem to indicate that the inhibition of germination by a high concentration of smoke solution is due to other inhibitory compounds (i.e. not the same compound(s) that are involved in the promotion of germination). Therefore, it is likely that different chemicals, or groups of chemicals, are responsible for the inhibition and promotion of germination by smoke. This supports the idea of an antagonistic interaction of compounds, in which the promoter cannot be removed once applied, but the inhibitory compounds can be removed. At high concentrations of aqueous smoke dilutions, it would seem that the inhibitor(s) override the promotion of germination. PRESTON and BALDWIN (1999) have also investigated the positive and negative signals that are involved in the regulation of germination in the

post-fire annual, *Nicotiana attenuata*. It was proposed that the combined stimulatory effect of smoke and the inhibitory effect of signals from unburned litter enable *N. attenuata* to identify its germination niche.

DE LANGE and BOUCHER (1993b) previously put forward the suggestion that the germination stimulus is recognized by the embryo but that germination occurred only when the environmental conditions were suitable for seedling establishment. In terms of the ecology of a post-fire environment, the presence of inhibitory compounds, which could be leached with sufficient rainfall, would provide a mechanism for preventing germination until conditions were more suitable.

4.4 SUMMARY

- Grand Rapids lettuce seeds were pulse-treated for different durations up to 6 h with 1:1000 and 1:100 dilutions of a smoke extract
- In general, germination increased with increased duration of smoke treatment up to an optimum
- Seeds treated with a higher concentration of smoke solution required a shorter treatment time to attain maximum germination
- Seeds that were pulse-treated with 1:100 dilution of smoke extract showed improved germination, in comparison to seeds that were continuously treated with the same solution
- These pulse-treatments indicated that there is a required threshold level of the active compound in the seeds
- Grand Rapids lettuce seeds were pulse-treated with a 1:100 dilution of smoke extract for 1 h and rinsed either before or after storage at 4 °C for 14 days
- Following a 14 day storage period, smoke-treated seeds showed improved germination when re-imbibed and incubated in the dark at 25 °C
- Retention of the germination cue by the seeds, and the reversal of the inhibitory effects of high smoke concentrations by rinsing, suggest a dual role for the regulation of germination by smoke solutions

5 Is Nitric Oxide the Active Compound in Smoke?

Just when you think you see the whole picture
of life clearly, the channel changes!

~ Unknown ~

5.1 INTRODUCTION

Nitrogen oxides (nitrites [NO_2^-], nitrates [NO_3^-] and nitric oxide [NO]) are known to play important roles in the germination of many species (see **Section 2.1.3.2**). For example, THANOS and RUNDEL (1995) reported that nitrates stimulated the germination of two post-fire annuals, *Emmenanthe penduliflora* and *Phacelia grandiflora*, and concluded that the increased levels of nitrate and ammonium following a fire were important factors for inducing germination in these species. In another study, nitrogen oxides have been shown to induce 100% seed germination of *E. penduliflora* seeds in a manner similar to smoke (KEELEY and FOTHERINGHAM, 1997). NO_2 was more stimulatory than NO_x ($NO + NO_2$) and induced germination both directly and indirectly. Furthermore, it was estimated that wildfires generate large amounts of nitrogen oxides from the combustion of organic matter to trigger the germination of *E. penduliflora*. In a further study by KEELEY and FOTHERINGHAM (1998a), investigating the mechanism of smoke-induced seed germination, the post-fire flora of Californian chaparral species did not appear to be triggered by nitrate, although NO_2 at concentrations present in biomass smoke was effective at inducing germination. Consequently, KEELEY and FOTHERINGHAM (1998a, 1998b, 2000) concluded that nitrogen oxides in smoke are most likely responsible for stimulating seed germination. PRESTON, BECKER and BALDWIN (2004), however, could not detect NO_2^- in aqueous smoke solutions derived from burned cellulose or wood, although these solutions effectively promoted germination of *E. penduliflora* and *Nicotiana attenuata*.

Nitric oxide is a small free radical gas that is water and lipid soluble. It can exist as three

interchangeable species and has a relatively short half-life, in the order of a few seconds (NEILL, DESIKAN and HANCOCK, 2003). The action of NO as a bioactive molecule in mammalian cells is well known (GOW and ISCHIROPOULOS, 2001). More recently, however, NO has emerged as a key signalling molecule in plants (DURNER and KLESSIG, 1999; LAMATTINA, GARCÍA-MATA, GRAZIANO and PAGNUSSAT, 2003). It is recognized to be an important signal and effector molecule during development and in adaptive plant responses, supporting the idea of NO as a versatile molecule with variable functions, as seen in animal systems. Furthermore, it is also known to function as a pro-oxidant as well as an antioxidant (LAMATTINA, GARCÍA-MATA, GRAZIANO and PAGNUSSAT, 2003). **Table 5.1** summarises a few examples of biological effects of NO in plants, following treatments with NO-releasing compounds.

Table 5.1: Examples of the effects observed with treatments of NO-releasing compounds.

Biological action	Reference
Induced phytoalexin accumulation in potato tuber tissues	NORITAKE, KAWAKITA and DOKE, 1996
Induced root tip expansion of maize root segments	GOUVÊA, SOUZA, MAGALHÃES, and MARTINS, 1997
Promoted germination of *Paulownia tomentosa* seeds	GIBA, GRUBIŠIĆ, TODOROVIĆ, SAJC, STOJAKOVIĆ, KONJEVIĆ, 1998
Promoted germination of Grand Rapids lettuce seeds Promoted de-etiolation in wheat seedlings Inhibited hypocotyl and internode elongation of Grand Rapids lettuce	BELIGNI and LAMATTINA, 2000
Reduced paraquat toxicity in rice leaves	HUNG, CHANG and KAO, 2002
Induced adventitious root development in cucumber hypocotyl cuttings	PAGNUSSAT, SIMONTACCHI, PUNTARULO and LAMATTINA, 2002
Inhibited senescence of deyhydrated and PEG-treated rice leaves	CHENG, HSU and KAO, 2002
Promoted seed germination and root growth of lupin	KOPYRA and GWÓŹDŹ, 2003
Promoted seed germination of dormant *Arabidopsis* and barley	BETHKE, GUBLER, JACOBSEN and JONES, 2004

In a study by BELIGNI and LAMATTINA (2000), it was reported that the NO-donor sodium nitroprusside (SNP), at a concentration of 100 µM, promoted the germination of Grand Rapids lettuce seeds in the dark. The NO specific scavenger 2-(4-

carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potassium (c-PTIO) counteracted the effect of NO and NO-donor compounds, and markedly inhibited the SNP- and *S*-nitroso-*N*-acetylpenicillamine (SNAP)-stimulated germination of Grand Rapids lettuce seeds at a concentration of 100 µM (BELIGNI and LAMATTINA, 2000). In this same study, nitrite and nitrate, two NO-decomposition products, were ineffective in stimulating the germination of Grand Rapids lettuce seeds. Similarly, the NO-releasing compounds SNP and SNAP stimulated the germination of *Paulownia tomentosa* seeds at concentrations between 100 and 1000 µM (GIBA, GRUBIŠIĆ, TODOROVIĆ, SAJC, STOJAKOVIĆ and KONJEVIĆ, 1998).

The aim of the following experiments were: (1) to investigate the effect of N-tert-butyl-α-phenylnitrone (PBN) and SNP, both NO-releasing compounds, on the germination of Grand Rapids lettuce seeds; and (2) to determine whether c-PTIO could reduce the effect of smoke-stimulated germination of Grand Rapids lettuce seeds.

5.2 MATERIALS AND METHODS

For each treatment, four replicates of 25 seeds were incubated in the dark at 25 °C for 24 h in 65 mm plastic Petri dish fitted with two sheets of Whatman No. 1 filter paper moistened with 2 mL of test solution. **Section 3.2.1** describes the lettuce seeds and smoke extract used. The chemicals PBN (Sigma) and SNP (Sigma) were used as NO-donors, and c-PTIO (Sigma) was used as NO-scavenger.

Seeds were treated with 10mM, 1000, 100 or 10 µM of PBN and SNP, and mixtures containing 1:1000 smoke solution and 1000, 100 or 10 µM c-PTIO. The solutions were prepared immediately before application to the seeds. To determine any inhibition by the PBN and SNP test solutions (including 10 mM), a set of seeds were exposed to 10 min red light (660 nm = 1.8 µmol m^{-2} s^{-1}; PAR value of 26.4 µmol m^{-2} s^{-1}) after 2 h imbibition in the dark. Distilled water was used as a control, and a 1:1000 dilution of the smoke solution was used as a standard treatment in each set of experiments. Each experiment was repeated twice.

5.3 RESULTS AND DISCUSSION

Seeds treated with water alone showed minimal levels of germination in the dark (**Table 5.2**). At a concentration of 10 mM, PBN reduced germination below levels observed with the water control, even when treated with 10 min of red light. Treatment with SNP at 10 mM, however, did not reduce germination of the red light-treated seeds, but the radicles appeared to be slightly discoloured and had shorter root hairs in comparison to the water control.

Table 5.2: The effect of PBN, SNP, c-PTIO and a smoke solution on the germination of Grand Rapids lettuce seeds after 24 h in the dark at 25 °C.

Treatment	Germination (%)[a]
H_2O	21.7 ± 11.0
Smoke 1:1000	86.6 ± 11.1 *
10 µM PBN	18.0 ± 18.4
100 µM PBN	14.7 ± 13.1
1000 µM PBN	12.0 ± 7.2
10 µM SNP	10.5 ± 9.6
100 µM SNP	14.0 ± 11.5
1000 µM SNP	12.5 ± 7.2
1:1000 Smoke + 10 µM c-PTIO	81.0 ± 11.3 *
1:1000 Smoke + 100 µM c-PTIO	80.0 ± 13.9 *
1:1000 Smoke + 1000 µM c-PTIO	77 ± 19.1 *

[a]Asterisks denote a significant ($p < 0.05$) difference from the water control; error bars indicate SD.

At lower concentrations (10, 100 and 1000 µM), both the PBN and SNP showed no inhibition of germination (red light treatment, results not shown), but did not enhance the germination of seeds incubated in the dark, contrary to the observations of BELIGNI and LAMATTINA (2000). It is possible that the use of different seed batches could account for these discrepancies in results. However, a treatment of 1:1000 smoke solution stimulated germination above 80%, suggesting that factors in the smoke, other than NO may be responsible for the enhanced germination. In combination with 1:1000 aqueous smoke solution, c-PTIO did not show any inhibition of the stimulatory effect of the

smoke, further indicating that NO may not be responsible for the germination induced by smoke solutions. It is possible that traces of NO in smoke and smoke solutions do play a role in the signalling of germination, as seen in the number of species that respond to treatments of NO-releasing and other nitrogenous compounds. However, the inability of the two NO-donors used to enhance germination, and the germination of seeds by aqueous smoke solution in the presence of c-PTIO, a NO scavenger, suggest that smoke-induced germination of Grand Rapids lettuce seeds does not require NO.

In a similar study by PRESTON, BECKER and BALDWIN (2004), it was demonstrated that NO was not responsible for the smoke-stimulated germination observed in *Nicotiana attenuata* and *Emmenanthe penduliflora*. Dormant seeds of *E. penduliflora* and *N. attenuata* were treated with smoke solutions, or the NO-donors SNP and SNAP. The smoke solutions promoted seed germination in both species, although no detectable quantities of NO_2^- were found in the solutions. The treatments with the NO-donors had no effect on germination, although 100 µM of SNP showed a slight promotion of germination of *N. attenuata*, in comparison to the water control. This was, however, well below the germination levels observed with treatments with smoke solutions. Thus, PRESTON, BECKER and BALDWIN (2004) also concluded that NO is not the component in smoke responsible for inducing germination in the smoke-stimulated species *E. penduliflora* and *N. attenuata*.

The results presented here were obtained prior to the identification of 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one (see **Chapter 6**). They supported the idea that NO was not the main component of smoke responsible for signalling germination, and that another highly active germination factor was present in plant-derived smoke (VAN STADEN, JÄGER, LIGHT and BURGER, 2004).

5.4 SUMMARY

- Grand Rapids lettuce seeds were treated with the NO-donors PBN or SNP (10, 100 or 1000 µM)
- These NO-donors did not promote the germination of Grand Rapids lettuce seeds
- Grand Rapids lettuce seeds were treated with the NO scavenger c-PTIO (10, 100 or 1000 µM) in combination with a standard smoke solution (1:1000 dilution)
- No reduction of the stimulatory effect of the smoke was observed in combination with the c-PTIO
- This indicates that NO is not likely to be responsible for the promotion of germination of the Grand Rapids lettuce seeds by smoke solutions

6 Isolation of the Germination Cue from Smoke

They asked me how I knew, my compound was true...
I of course replied, GC and NMR, cannot be denied
They said someday you'll find, that chemical sublime...
When you see the seeds, waking from their dreams
Smoke gets in your eyes!

~ Words adapted from original lyrics by Otto Harbach ~

6.1 INTRODUCTION

One of the greatest hurdles of research in the topic of smoke-stimulated seed germination has been the difficulty in isolating and identifying the active chemical factor(s) present in plant-derived smoke. For a number of years, our research group has attempted to isolate and identify the principal germination factor(s) in smoke (VAN STADEN, DREWES and BROWN, 1995; VAN STADEN, DREWES and JÄGER, 1995). The characterization of a novel, highly active compound is thus a major step forward to furthering research in this area.

6.1.1 The chemical nature of the germination cue

As discussed in **Section 2.3.1**, smoke is known to contain several thousand compounds. For a long time the identity of the germination cue was unknown, although smoke products have been successfully used for their germination promoting properties (see **Section 2.3.3.2**). Prior to the isolation and identification of an active compound from smoke, described in this chapter, it was known that the germination cue(s) were water soluble chemical(s) that were thermostable, long-lasting in solutions, and highly active at very low concentrations (BALDWIN, STASZAK-KOZINSKI and DAVIDSON, 1994; VAN STADEN, BROWN, JÄGER and JOHNSON, 2000).

Although there have been a large number of studies on the chemical makeup of smoke, particularly related to cigarette smoke and food flavouring smoke, analysis of such highly complex mixtures is extremely difficult. In a study on the effect of moisture content on the composition of liquid smoke production, GUILLÉN and IBARGOITIA (1999) detected 148 compounds in the dichloromethane extracts of the liquid smoke produced. JAKAB, LIU and MEUZELAAR (1997) demonstrated the difficulty of separating smoke components of low molecular weights by on-line gas chromatography-mass spectrometry (GC-MS). Furthermore, although a number of compounds are present in smoke and liquid smoke preparations in relatively large quantities, there are many more compounds which are present in minute amounts.

6.1.2 Isolation of the active components

Following the initial reports on smoke-stimulated seed germination in the early 1990s, various research groups worldwide attempted to isolate and characterize chemicals present in plant-derived smoke responsible for the promotion of germination by smoke. BALDWIN, STASZAK-KOZINSKI and DAVIDSON (1994) identified 71 compounds in active fractions of smoke by GC-MS and atomic absorption (AA) spectrometry, and tested a total of 233 compounds using seeds of *Nicotiana attenuata*. None of these compounds, however, promoted germination.

Chromatographic separation of two different smoke extracts, from burned fynbos or burned *Themeda*, showed one major peak of germination activity with a similar retention time from the non-polar fraction (VAN STADEN, DREWES and BROWN, 1995). In addition, some smaller peaks of activity were observed. Germination activity was tested using achenes of *Syncarpha vestita* (fynbos plant) and caryopses of *Themeda triandra* (climax grass). Similar results were also observed using Grand Rapids lettuce seeds. The lettuce seeds were more suitable for bioactivity-guided fractionation than the *Syncarpha* achenes, which required 20 days or longer to show an optimal response. The study by VAN STADEN, DREWES and BROWN (1995) also demonstrated that ethylene was probably not responsible for the germination activity, because it is unlikely that it would still be present in the active fractions following the various chromatographic procedures.

Continuing from the above-mentioned study, VAN STADEN, DREWES and JÄGER (1995) identified seven compounds present in both *Passerina vulgaris* and *Themeda triandra* smoke extracts. Four of the compounds (available commercially) were tested in the lettuce seed bioassay at concentrations from 10^{-4} to 10^{-15} M. However, none were found to be active.

JÄGER, RABE and VAN STADEN (1996) investigated a smoke extract from *Themeda triandra* and a liquid food-flavouring concentrate, using Grand Rapids lettuce seed to detect germination activity. Chromatographic separation of these two extracts, using thin layer chromatography (TLC), semi-preparative high-performance liquid chromatography (HPLC), and analytical HPLC, indicated that the germination activity was detected in the same fractions. Although bioassay-guided fractionation has led to one major peak of activity, there is some chromatographic evidence indicating that there is more than one active component in smoke that promotes seed germination (BALDWIN, STASZAK-KOZINSKI and DAVIDSON, 1994; VAN STADEN, DREWES and BROWN, 1995).

ADRIANSZ, RUMMEY and BENNETT (2000) reported the monoterpene 1,8-cineole to be a germination cue for *Lactuca sativa* L. cv. Grand Rapids partitioned from smoke water. However, FLEMATTI, GHISALBERTI, DIXON and TRENGOVE (2001) were unable to obtain a positive germination response with 1,8-cineole, and suggested that other factors may have resulted in the germination enhancement observed by the Adriansz research group.

It was estimated that less than 1 pg of the active chemical is needed per seed (BALDWIN, STASZAK-KOZINSKI and DAVIDSON, 1994). Thus, the difficulty in isolating the active component(s) from aqueous smoke extracts was partly due to the large number of compounds present in the extract, which was further complicated by the very low concentration of the active compound(s) needed to elicit a germination response.

6.2 MATERIALS AND METHODS

6.2.1 Isolation of the germination promoter

The principal germination cue was isolated through bioactivity-guided fractionation using the lettuce seed bioassay (see **Section 3.2.1**). Smoke-saturated water (20 L), derived from burned *Passerina vulgaris* Thoday and *Themeda triandra* L., were concentrated under vacuum to 2 L. This concentrate was exhaustively partitioned against dichloromethane. The combined organic extracts were washed six times with 1% (w/v) aqueous NaOH, followed by three washings with water to neutral pH. The extract (9.8 g) was subjected to vacuum liquid chromatography (VLC) using a 5 x 27cm column packed with 200 g silica gel 60 (Merck, 0.040-0.063 mm) and eluted with a hexane:ethyl acetate gradient (hexane proportions: 100, 85, 80, 75, 70, 65, 60, 50, 40, 30, 20, 10, 0% (v/v); 400 mL of each mixture). Fractions with germination stimulating activity were eluted in 70:30 and 65:35 hexane:ethyl acetate (v/v).

The two fractions from the VLC were combined and then fractionated on a Sephadex LH-20 column (90 x 2.5cm) eluted with 35% ethanol at 15 mL h^{-1}. Activity eluted between 560-630 mL. This active material was concentrated under vacuum and the aqueous residue partitioned against dichloromethane to recover the active material.

Further purification was achieved by repeated reversed-phase HPLC (60 runs) on a C_{18} column (Haisil 300 C_{18}, 5µm, 250 x 10mm, Higgins Analytical) with 30% methanol as mobile phase at 2 mL min^{-1}. A Spectra System P4000 pump and UV6000LP photodiode array detector (Thermo Separation Products) were used. The active constituent eluted at 20 to 21min. The methanol was allowed to evaporate at room temperature and the active fractions combined and partitioned against equal volumes of dichloromethane yielding 3.1 mg of the target compound.

6.2.2 GC-MS analysis of the active constituent

Aliquots of 1-2 µL of the active HPLC fraction were subjected to preparative capillary

gas chromatographic separation on a 4000 series model Carlo Erba gas chromatograph (4000 Series Model HRGC) fitted with an on-column injector and a 40 m x 0.3 mm glass capillary column coated with 0.375 µm of OV-1701 as stationary phase. A capillary effluent splitter, which allowed 10% of the column effluent to flow to the detector and 90% to the collection device, was made in-house.

To avoid band broadening in space, expected to result from the injection of samples containing highly polar solvents, the column was connected to a 3 m retention gap. Fractions were collected manually. Preparative capillary GC is problematic because some of the effluent organic material can be lost due to aerosol formation. To circumvent this problem, fractions were collected in methanol or dichloromethane in small conical sample vials.

Another problem is the cross-contamination of fractions due to the condensation of organic material at the point where fractions from a hot capillary are collected in a cold solvent. To solve this problem, each fraction was collected using a clean glass exit capillary, which was connected in a "press-fit" fashion to the fused silica GC column.

All fractions were tested for germination activity and the active fraction was subjected to GC-MS analysis on both more and less polar capillary columns to determine the purity of the isolated active material. The preparative separations were repeated 20 times with collection of the active fraction after which the preparative separations were repeated first on a 40 m x 0.3 mm glass capillary column coated with 2.5 µm of the apolar phase PS-255 and finally on a 30 m x 0.32 mm fused silica column coated with 0.25 µm Carbowax 20M. According to GC and GC-EIMS analyses (Carlo Erba QMD1000 quadrupole instrument, Carbowax 20M column), this procedure produced the active constituent in a highly pure state.

The isolated compound had the same mass spectrum as the major constituent of the active material isolated by HPLC. This material was subjected to high resolution mass spectral measurements (MicroMass Autospec-TOF instrument; calculated for $C_8H_6O_3$ 150.0317; found 150.0316).

6.2.3 Structure elucidation

Infrared (IR) spectrum was recorded on a Perkin Elmer 1600 instrument, ($CHCl_3$): (C=O) 1743 cm^{-1}.

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 600 or a Bruker Avance 400 spectrometer (proton frequency 600.13 and 400.13 MHz, respectively) at 25 °C, using $CDCl_3$ as solvent and tetramethylsilane as internal standard. NOESY spectrum was obtained with mixing time of 300. The gHMBC and gHSQC spectra were optimized for $^{n}J_{C,H}$ = 7.7 Hz and $^{1}J_{C,H}$ = 140 Hz, respectively. ^{1}H NMR (600.13 MHz, $CDCl_3$) δ 7.44 (1H, s, H-7), 7.32 (1H, d, *J* = 5.5 Hz, H-5), 6.51 (1H, d, *J* = 5.5 Hz, H-4), 1.93 (3H, s, H-8). ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 7.70, 100.40, 103.46, 126.79, 139.73, 142.31, 147.97, 171.25.

6.3 RESULTS AND DISCUSSION

6.3.1 Isolation and structure elucidation

Following liquid-liquid partitioning of the smoke-saturated water, the extract was fractionated using VLC. The VLC separation yielded 13 fractions, two of which showed germination stimulating activity (70:30 and 65:35 hexane:ethyl acetate, v/v). These two fractions were combined and subjected to column chromatography on Sephadex LH-20 followed by reversed-phase HPLC.

GC-MS analysis showed that the active fraction contained very small quantities of impurities. It was, however, sufficiently pure for NMR and IR spectroscopy. Further purification of the active material by preparative capillary GC on three columns coated with stationary phases having different polarities yielded a fraction containing a single compound. This compound had high germination stimulating activity and had the same mass spectrum as the major constituent of the HPLC-isolated material mentioned above (**Figure 6.1**). Once again, the preparative GC isolation of the germination stimulant rules out the possibility that nitric oxide could be the active smoke principal (see

Chapter 5). It also rules out the possibility that any impurities could be responsible for the germination stimulating activity of the HPLC-isolated material.

Electrospray ionisation-mass spectrometry (ESI-MS) of the active fraction, isolated by HPLC, gave mass spectra having an abundant pseudomolecular ion at *m/z* 151 ($M+H^+$).

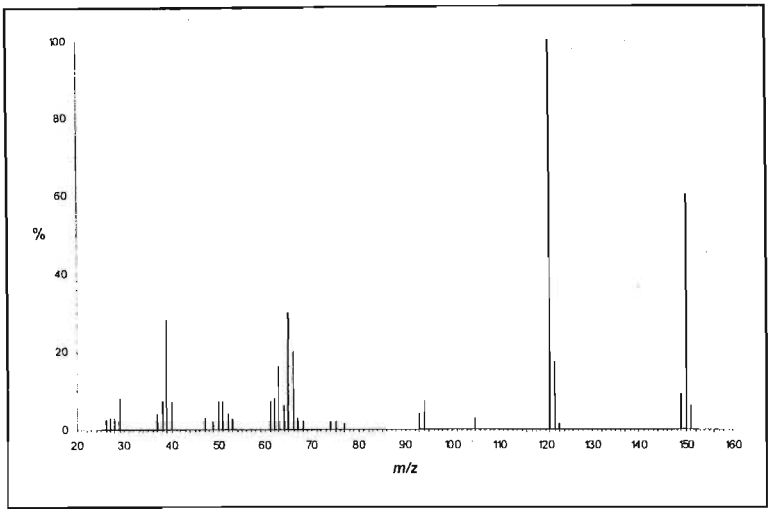

Figure 6.1: Mass spectrum of the germination promoter, 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one, isolated from plant-derived smoke.

High resolution electron impact mass spectral measurements (HR-EIMS) of the gas chromatographically isolated active constituent showed that this compound has the molecular formula $C_8H_6O_3$. The possible presence of an α,β-unsaturated γ-lactone was indicated by a carbonyl stretch at 1743 cm^{-1} in the IR spectrum and a signal at 171 ppm in the ^{13}C NMR spectrum. A full assignment of data obtained from 1D ^1H and ^{13}C NMR, as well as 2D homonuclear and heteronuclear NMR experiments, showed the active constituent to be 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one (**Figure 6.2**). This was a previously unknown compound.

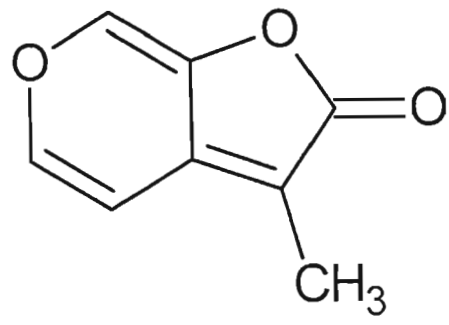

Figure 6.2: Chemical structure of 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one.

6.3.2 Germination activity

The isolated compound proved to be a highly active germination promoter in the lettuce seed bioassay, where it was active at concentrations as low as 10^{-9} M (**Figure 6.3**).

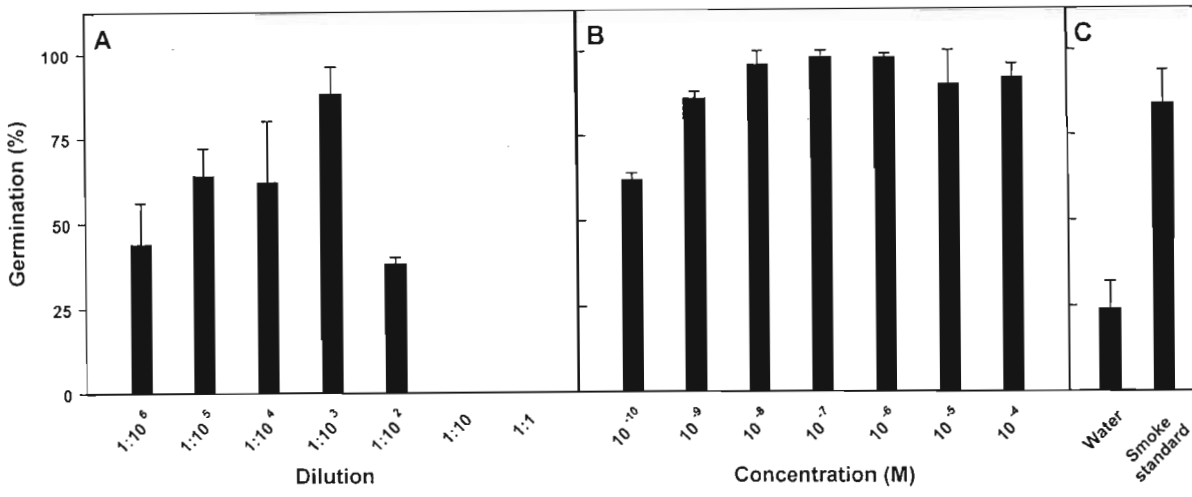

Figure 6.3: Germination of Grand Rapids lettuce seeds treated with (A) different dilutions of smoke extract, (B) 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one at different concentrations, or (C) distilled water and standard smoke solution at a 1:1000 dilution (controls) after 24 h in the dark at 25 °C. Error bars indicate SE.

The compound showed germination activity over a wide range of concentrations and did not inhibit seed germination at high concentrations (**Figure 6.3B**), as was observed with crude smoke extracts (**Figure 6.3A**; DREWES, SMITH and VAN STADEN, 1995). This indicates that the inhibitory effect observed with more concentrated and non-purified

smoke water is due to the presence of inhibitory compounds in the smoke water. A dual role for smoke, both stimulatory and inhibitory, in seed germination has been reported (LIGHT, GARDNER, JÄGER and VAN STADEN, 2002) and is discussed in **Section 4.3.3**.

At the time of preparation of this thesis chapter and corresponding manuscript, a paper appeared in *Science* in which the same compound was isolated from smoke produced by burning cellulose (FLEMATTI, GHISALBERTI, DIXON and TRENGOVE, 2004). Cellulose is a ubiquitously occurring plant compound which was previously reported to produce a germination stimulant (BALDWIN, STASZAK-KOZINSKI and DAVIDSON, 1994; JÄGER, LIGHT and VAN STADEN, 1996). Using GC-MS, FLEMATTI, GHISALBERTI, DIXON and TRENGOVE (2004) also confirmed the presence of this compound in plant-derived smoke. They showed that this compound significantly improved the germination of Australian indigenous plants, and smoke responsive South African and North American species. FLEMATTI, GHISALBERTI, DIXON and TRENGOVE (2005) have also subsequently reported on the synthesis of 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one, in a three-step process, from pyromeconic acid.

It is of additional interest that the natural product (+)-strigol (**Figure 6.4**), and related strigolactones, contain a butyrolactone moiety, as found in 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one (HUMPHREY and BEALE, 2006).

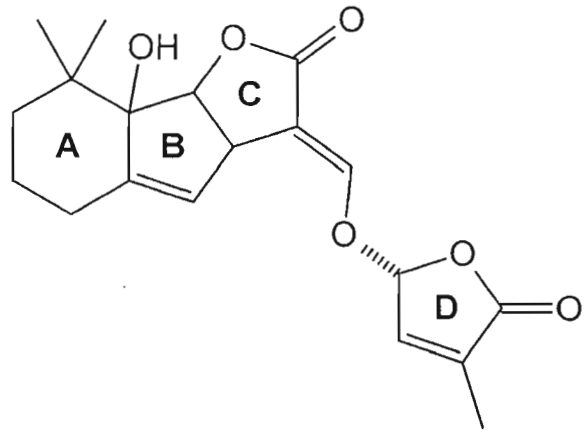

Figure 6.4: Chemical structure of (+)-strigol, showing the butyrolactone moiety (the D-ring).

Strigolactones are secreted by the roots of numerous plants and are highly active germination stimulants of *Striga* (witchweed) and *Orobanche* (broomrape), parasitic weeds which will only germinate after induction by minute amounts of a chemical signal from the root exudates of their host plants (MATUSOVA, RANI, VERSTAPPEN, FRANSSEN, BEALE and BOUWMEESTER, 2005). These compounds are active at very low concentrations, not unlike the smoke-derived germination cue. For example, an application of a 10^{-11} M solution of (+)-strigol results in over 50% germination of *Striga lutea* seeds (COOK, WHICHARD, WALL, EGLEY, COGGON, LUHAN and McPHAIL, 1972).

Studies on the structure-activity relationship of strigolactones, using synthetic analogues of strigol, have shown that the lactone-enol ether (on the CD portion of the molecule; **Figure 6.4**) is primarily responsible for the biological activity of these compounds (MANGNUS and ZWANENBURG, 1992; WIGCHERT and ZWANENBURG, 1999). However, as is the case with smoke-stimulated germination, the mode of action of strigol remains "shrouded in mystery" (HUMPHREY and BEALE, 2006).

The identification of 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one from plant-derived smoke now opens the door to study its mode of action in more detail, and better understand the role of smoke in seed germination ecology. For example, attempts can be made to answer the question of how this molecule is capable of substituting for phytochrome effects in light-sensitive lettuce (DREWES, SMITH and VAN STADEN, 1995; see **Chapter 3**). Furthermore, as discussed in **Section 2.6**, this also has the potential to impact on agriculture, forestry and horticulture (LIGHT and VAN STADEN, 2004; VAN STADEN, SPARG, KULKARNI and LIGHT, 2006).

6.3.3 Recent studies with 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one

To date, a few reports on the effect of 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one have been published. Our research group has conducted various studies on germination and post-germination effects of 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one. Treatment of some vegetable crops with this compound has provided some remarkable results. Seeds of

tomato, okra and bean showed a significant improvement in seedling vigour in comparison to controls when treated with this compound (VAN STADEN, KULKARNI, SPARG and LIGHT, 2006). In a study on an important South African medicinal plant species, *Eucomis autumnalis*, treatment of seeds with the smoke compound substituted for the dark and cold stratification conditions required for germination (KULKARNI, SPARG and VAN STADEN, 2006).

The Australian research group has also extended their research on this compound. For example, the effect of 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one on light-mediated germination of Australian Asteraceae species has been investigated (MERRITT, KRISTIANSEN, FLEMATTI, TURNER, GHISALBERTI, TRENGOVE and DIXON, 2006). For five of the seven species tested, the compound increased germination at suboptimal light conditions, and in the dark, to a level equal to, or greater than, that obtained with smoke water. It is interesting to note, however, that for two of the species, the smoke compound did not overcome the light requirement.

6.4 SUMMARY

- Using bioactivity-guided fractionation, the principal germination cue from plant-derived smoke was isolated
- The compound structure was elucidated, using NMR spectroscopy, as 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one ($C_8H_6O_3$)
- Application of a wide range of concentrations of the compound, and at a concentration as low as 10^{-9} M, stimulates the germination of Grand Rapids lettuce seeds in the dark
- This compound is also present in smoke derived from burning cellulose

6.5 COLLABORATION

The research presented in this chapter is the culmination of several years of collaborative research between Prof. J. van Staden, Prof. A.K. Jäger, Prof. B.V. Burger and myself. The GC-MS, ESI-MS and IR experimental work was conducted by Prof. B.V. Burger (University of Stellenbosch). Dr D. Stærk (The Danish University of Pharmaceutical Sciences) recorded the NMR spectra.

7 Formation of the Germination Promoter from Carbohydrates and Amino Acids

The man who moves a mountain
begins by carrying away small stones.

~ Chinese proverb ~

7.1 INTRODUCTION

As discussed in **Chapter 6**, the isolation and characterization of a highly active germination promoter from plant-derived smoke is an important breakthrough in research related to smoke-stimulated seed germination. In an attempt to produce a greater yield of the active constituent, and to explore the formation of the compound, we investigated different reactions between carbohydrates and amino acids.

7.1.1 Common constituents for smoke production

In some earlier studies on smoke-stimulated germination, the effect of different sources of smoke, including liquid food-flavouring smoke, on seed germination was investigated (BAXTER, GRANGER and VAN STADEN, 1995; JÄGER, LIGHT and VAN STADEN, 1996; JÄGER, RABE and VAN STADEN, 1996). A wide range of sources of plant material produced smoke that stimulates the germination of *Themeda triandra* (BAXTER, GRANGER and VAN STADEN, 1995) and Grand Rapids lettuce seeds (JÄGER, LIGHT and VAN STADEN, 1996; JÄGER, RABE and VAN STADEN, 1996). Results from these studies suggested that the germination-promoting compound(s) is produced from commonly occurring plant constituents. Furthermore, chromatographic purification of aqueous smoke extracts from fynbos material and *T. triandra*, as well as a commercial food-flavourant, produced results supporting the notion of a common active compound (VAN STADEN, DREWES and BROWN, 1995; VAN STADEN, DREWES and JÄGER, 1995; JÄGER, RABE and VAN STADEN, 1996).

The initial moisture content, combustion rate and temperature at which plant material burns influences the final mixture of products formed, and may account for slight differences in germination activity observed in the use of different source material in producing smoke and aqueous smoke extracts (also see **Section 2.3**; BAXTER, GRANGER and VAN STADEN, 1995). Furthermore, as discussed in **Chapter 4**, there is evidence to suggest that inhibitory compounds are also present in smoke which would interfere with the stimulatory effect observed (BALDWIN, STASZAK-KOZINSKI and DAVIDSON, 1994; LIGHT, GARDNER, JÄGER and VAN STADEN, 2002).

7.1.2 Heating plant material

The effect of germination stimulation is not limited to extracts produced from smoke only. As discussed in **Section 2.1.3.2**, the exposure of seeds directly to charred wood from a variety of sources and aqueous extracts of charred wood and plant material also stimulates germination of a number of chaparral species (KEELEY, MORTON, PEDROSA and TROTTER, 1985; KEELEY and PIZZORNO, 1986; KEELEY, 1987) and a fynbos species (BROWN, 1993b). Furthermore, KEELEY and PIZZORNO (1986) suggested that charring was not necessary and showed that the dry heating of *Adenostoma* wood at 175 °C for 30 min produced compounds, which promoted seed germination in chaparral species. In a study by JÄGER, LIGHT and VAN STADEN (1996), heating dry *T. triandra* leaves at a range of temperatures showed that the active compound(s) was produced between 160 and 200 °C. At higher temperatures, germination activity was no longer observed, probably because of volatilization of the active component(s).

It has been suggested that cellulose and hemicelluloses are the most likely common components for the production of active compound(s) in smoke (KEELEY and PIZZORNO, 1986; JÄGER, LIGHT and VAN STADEN, 1996). KEELEY and PIZZORNO (1986) observed increased germination of two chaparral species with an extract from crude lignin, although some purified lignin compounds did not increase germination. They tested a variety of extracts from heated cellulose and hemicelluloses, and observed some increase in germination with extracts of heated xylan and glucuronic

acid. However, these treatments were not as effective as the crude extracts from charred wood. JÄGER, LIGHT and VAN STADEN (1996) did not observe any improved germination of light sensitive lettuce seeds with extracts of heated glucuronic acid, starch, glucose, and galactose. However, extracts from heated agar and cellulose did stimulate germination. This study also presented chromatographic evidence suggesting that the same active compound(s) is formed from commonly occurring plant constituents such as cellulose. Subsequently, FLEMATTI, GHISALBERTI, DIXON and TRENGOVE (2004) isolated the germination cue 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one from burned filter paper (a cellulose product).

7.1.3 Maillard reactions

It is well established that many classic Maillard reaction products are also formed by pyrolytic reactions (BALTES and BOCHMANN, 1986). These products, formed from commonly occurring organic compounds, may be responsible for the germination stimulation observed with smoke, aqueous smoke extracts, charred wood extracts and extracts from heated plant material.

The Maillard reaction, named after the French scientist Louis-Camille Maillard, is a non-enzymatic browning reaction caused by the condensation of an amino group and a reducing sugar (ERIKSSON, ASP and THEANDER, 1981). In food, the reaction commonly occurs between amino acids (free and peptide-bound) and reducing sugars during cooking and food preservation. This results in browning and the production of aromas and flavours, but can also result in certain undesirable changes.

The classic Maillard reaction comprises reactions of aldehydes, ketones and reducing sugars with amines, amino acids, peptides and proteins (MAURON, 1981). It is an extremely complex series of reactions which can be divided into three stages: early Maillard reactions, advanced Maillard reactions and final Maillard reactions. The first stage corresponds to steps without browning, the second to a myriad of reactions which lead to volatile and soluble substances, and the third stage of reactions result in insoluble brown polymers (melanoidins) (MAURON, 1981). The early Maillard reactions are quite simple and have been chemically well-defined. However, the later reactions,

and particularly the formation of melanoidins, are not fully understood (MAURON, 1981).

The complexity of Maillard reactions arises from the large number of components having various functional groups, molecular sizes and linkages that are involved in these reactions (NURSTEN and O'REILLY, 1986). Furthermore, the course of the Maillard reaction is strongly influenced by reaction conditions such as temperature, duration of heating, moisture content of the reactants and pH (MAURON, 1981).

Thus, the aim of these experiments was to investigate the possible role of Maillard reactions of amino acids and sugars in the formation of the compound responsible for the stimulation of germination by smoke and aqueous smoke solutions. We examined the effect of water extracts, from the products of reactions between a variety of amino- and carbonyl-containing compounds, on the germination of light sensitive lettuce seeds. Extracts showing high levels of germination stimulation were further analysed using high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS).

7.2 MATERIALS AND METHODS

7.2.1 Instrumentation

HPLC was performed using a semi-preparative reversed-phase HPLC column (Haisil, 300 C_{18}, 5 µm, 250 mm x 10 mm, Higgins Analytical) with 30% MeOH as the mobile phase at 2 mL min^{-1}. A Spectra System P4000 pump and UV6000LP photodiode array detector (Thermo Separation Products) were used.

Exploratory GC analyses were carried out with a Carlo Erba 5300 gas chromatograph equipped with a flame ionization detector, Grob split-splitless injector, and a glass capillary column (40 m x 0.3 mm) coated with PS-089-OH (DB-5 equivalent) at a film thickness of 0.25 µm. All analyses were done with hydrogen as a carrier gas at a linear velocity of 50.0 cm s^{-1}, determined by injecting methane at a column temperature of 40 °C. The injector was operated at 220 °C, and the flame ionization detector was operated at 280 °C. Samples were injected with a split ratio of 6:1, the analytes thermally

focussed on the column at ca. 27 °C, and analysed using a temperature program of 2 °C min^{-1} from 40 to 250 °C (hold).

Electron impact mass spectra (EIMS) were recorded at 70 eV on a Carlo Erba QMD 1000 GC-MS system, with VG Analytical Lab-Base software, using the column and conditions described above, except that helium was used as a carrier gas at a linear velocity of 31.4 cm s^{-1}, determined by injecting air at a column temperature of 40 °C. An interface temperature of 250 °C was used. The ion source temperature was set at 180 °C, and the pressure in the source housing was ca. 2 x 10^{-5} mmHg at a column temperature of 40 °C, decreasing to ca. 1 x 10^{-5} mmHg towards the end of the temperature program. A scan rate of 0.9 scan s^{-1}, with an interval of 0.1 s between scans, was employed.

7.2.2 Lettuce seed bioassay

All experimental solutions were tested for the germination promotion of light sensitive lettuce seeds as described in **Section 3.2**. All experiments were repeated at least twice.

7.2.3 Chemicals

The chemicals used were as follows: DL-α-alanine (BDH), L-arginine (Sigma), L-asparagine (anhydrous) (Sigma), DL-aspartic acid (BDH), bovine albumin (Sigma), casein (Merck), L-cysteine (Sigma), D-(-)-erythrose (Sigma), D-fructose (BDH), D-glucose (BDH), L-glutamic acid (Ashe Laboratories), DL-glutamine (BDH), DL-glyceraldehyde (Sigma), glycine (Merck), L-histidine hydrochloride (Saarchem), L-isoleucine (Sigma), DL-leucine (BDH), DL-lysine monohydrochloride (BDH), maltose (Associated Chemical Enterprises), DL-methionine (Sigma), DL-β-phenylalanine (BDH), L-proline (Sigma), DL-serine (BDH), D-ribose (Sigma), sucrose (BDH), DL-threonine (Nutritional Biochemicals Corporation), L-tryptophan (Sigma), L-tyrosine (Sigma), DL-valine (Sigma), and D-xylose (Sigma).

7.2.4 Reactions of proteins with glucose

Bovine albumin or casein (0.075 g) were intimately mixed with equal amounts (w/w) of glucose in a glass vial. The dry mixtures were heated at 180 °C for 30 min in a muffle furnace. The temperature and duration of heating for these preliminary experiments were chosen in accordance with the experiments of KEELEY and PIZZORNO (1986) and JÄGER, LIGHT and VAN STADEN (1996). The reaction products were allowed to cool slightly before the addition of 15 mL of distilled water. The resulting suspensions were allowed to stand overnight and then vigorously agitated for 30 min prior to filtration through Whatman No. 1 filter paper. The extracts were tested in the lettuce seed bioassay at dilutions of 1:1 and 1:10, and the results were compared to those obtained with the protein and glucose (0.15 g) heated individually.

7.2.5 Reactions of amino acids with glucose

Twenty commonly occurring amino acid compounds (3.75×10^{-4} mol) were mixed with glucose (7.5×10^{-4} mol) in a glass vial. The dry mixtures were heated at 180 °C for 30 min, after which 15 mL of distilled water was added to each. Each chemical (7.5×10^{-4} mol) was heated individually as a control. The extracts were prepared as above and tested in the lettuce seed bioassay at dilutions of 1:1, 1:10 and 1:100.

7.2.6 Variation of the amino acid-to-glucose ratio

Combinations of the aminocarbonyl compounds arginine, asparagine, or aspartic acid with glucose were prepared at the following molar ratios: 2:1, 3:2, 2:2, 2:3, and 1:2 (where 1 = 3.75×10^{-4} mol). The dry mixtures were heated at 180 °C for 30 min. The extracts were prepared as above and tested in the lettuce seed bioassay at dilutions of 1:1, 1:10, and 1:100.

7.2.7 Variation of reaction conditions

Mixtures of the aminocarbonyl compounds arginine, asparagine, or aspartic acid with

glucose were prepared at a 1:2 molar ratio (where 1 = 3.75 x 10^{-4} mol). The dry mixtures were heated at 160, 180, 200, and 220 °C for 5, 15, or 30 min after which 15 mL of distilled water was added to each vial. The extracts were prepared as above and tested in the lettuce seed bioassay at dilutions of 1:1, 1:10, and 1:100.

7.2.8 Reaction with different sugars

The aminocarbonyl compounds arginine, asparagine, aspartic acid, glycine, serine, tyrosine and valine were mixed with different sugars in a 1:2 molar ratio (where 1 = 3.75 x 10^{-4} mol). The sugars used were the monosaccharides DL-glyceraldehyde (triose), D-(-)-erythrose (tetrose), D-ribose (aldopentose), D-xylose (aldopentose), D-fructose (ketose), D-glucose (aldohexose), and the disaccharides maltose and sucrose. The dry mixtures were heated at 180 °C for 30 min. The extracts were prepared as above and tested in the lettuce seed bioassay at dilutions of 1:1, 1:10, and 1:100.

7.2.9 HPLC and GC-MS analysis

Extracts were prepared in the manner described previously by heating different combinations of amino acids and sugars. The combinations used included the following: arginine and glucose, arginine and lactose, asparagine and lactose, aspartic acid and glucose, glycine and glucose, and glycine and xylose. The water extracts obtained from the reactions were partitioned three times against equal volumes of dichloromethane. The dichloromethane phase was washed with 1% (w/v) aqueous NaOH, followed by washings with water to a neutral pH. The extracts were fractionated using HPLC as described above. The HPLC fractions (1 min) were collected and tested in the lettuce seed bioassay.

A mixture of larger quantities of glycine (3.75 x 10^{-3} mol) and xylose (7.5 x 10^{-3} mol) was heated at 220 °C in a custom-made apparatus (**Figure 7.1**). This apparatus allowed for the reaction of larger quantities of reactants, at a slightly higher temperature than that used in the examples described above, and for the collection of the volatile products in a 50 mL water trap (**Figure 7.1B**).

The water containing the volatile compounds was partitioned three times with 50 mL of dichloromethane. The dichloromethane phase was washed once with 1% aqueous sodium hydroxide, followed by a water wash. This dichloromethane extract was concentrated by allowing the dichloromethane to evaporate at room temperature. An aliquot of this extract was subjected to semi-preparative HPLC separation as described above. The active fractions from the HPLC were verified using the lettuce seed bioassay and extracted with dichloromethane for further analysis using GC-MS.

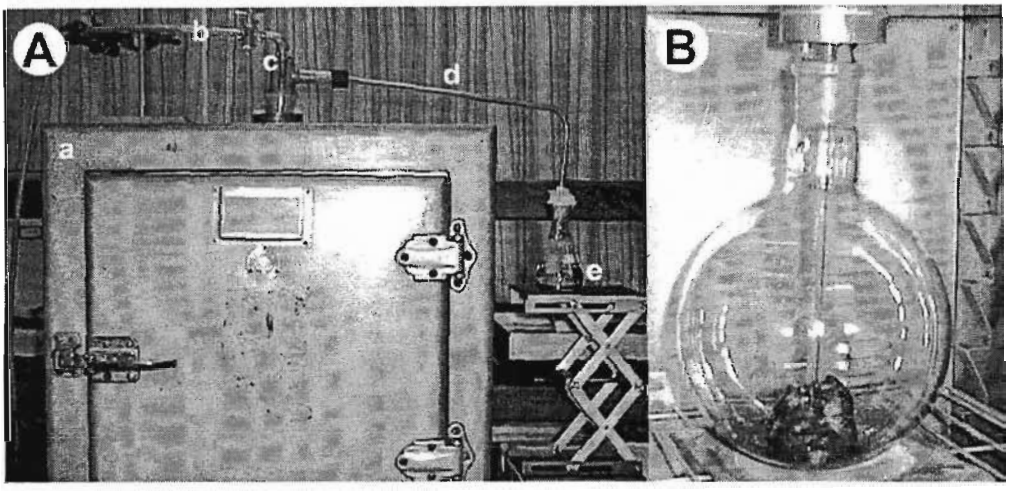

Figure 7.1: (A) Modified oven and apparatus for amino acid-sugar reactions: (a) oven with special opening in the top; (b) low pressure air inlet with stopcock valve; (c) inlet into oven; (d) outlet tube ending in glass frit; (e) collecting flask with 50 mL water. (B) Inside of oven showing reaction flask, inlet tube and insoluble product from reaction. (Nicknamed "Marnie's magic muffin maker").

The 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one previously isolated from a plant-derived smoke solution (**Chapter 6**; VAN STADEN, JÄGER, LIGHT and BURGER, 2004) was used in the GC-MS analysis as a standard for retention time and mass spectral comparison with the active fraction obtained from the glycine-xylose reaction.

7.3 RESULTS AND DISCUSSION

7.3.1 Reactions of proteins with glucose

Heating proteins or amino acids with sugars at temperatures between 160 and 220 °C for 30 min resulted in the production of a dark brown insoluble "honeycomb" substance

(40-50% of the starting material) (**Figure 7.1B**). Adding water to the products gave a solution containing the soluble components, although most of the product formed in the reaction was insoluble. Some solutions were clear, while others ranged from yellow or orange to dark brown in colour. The colour of the extract did not appear to correlate with germination activity. The germination activity was not related to the pH of the extracts which ranged from 2.14 to 8.87. At lower concentrations (e.g., 1:100 dilution), the pH differences were negligible.

The extracts prepared from heating purified proteins with glucose resulted in a marked germination response of the light sensitive Grand Rapids lettuce seeds when germinated in the dark at 25 °C (**Figure 7.2**). The proteins and glucose were heated individually in control experiments, which gave no germination in the lettuce seed bioassay.

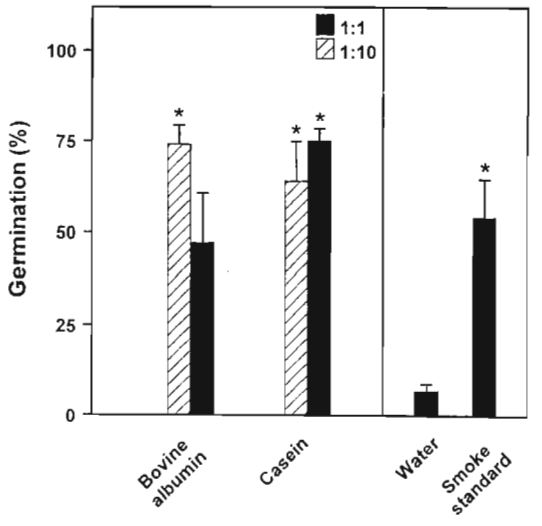

Figure 7.2: The effect of extracts, prepared from heating proteins with glucose at 180 °C for 30 min, on the germination of Grand Rapids lettuce seeds after 24 h in the dark at 25 °C. Asterisks denote a significant ($p < 0.05$) difference from the water control; error bars indicate SE.

7.3.2 Reactions of amino acids with glucose

Further experiments using common amino acids in combination with glucose were conducted. Germination of the lettuce seeds was enhanced by a number of extracts prepared using a 1:2 molar ratio of amino acid and glucose (**Figure 7.3**). The amino

acids and glucose were heated individually in control experiments, which gave no germination in the lettuce seed bioassay.

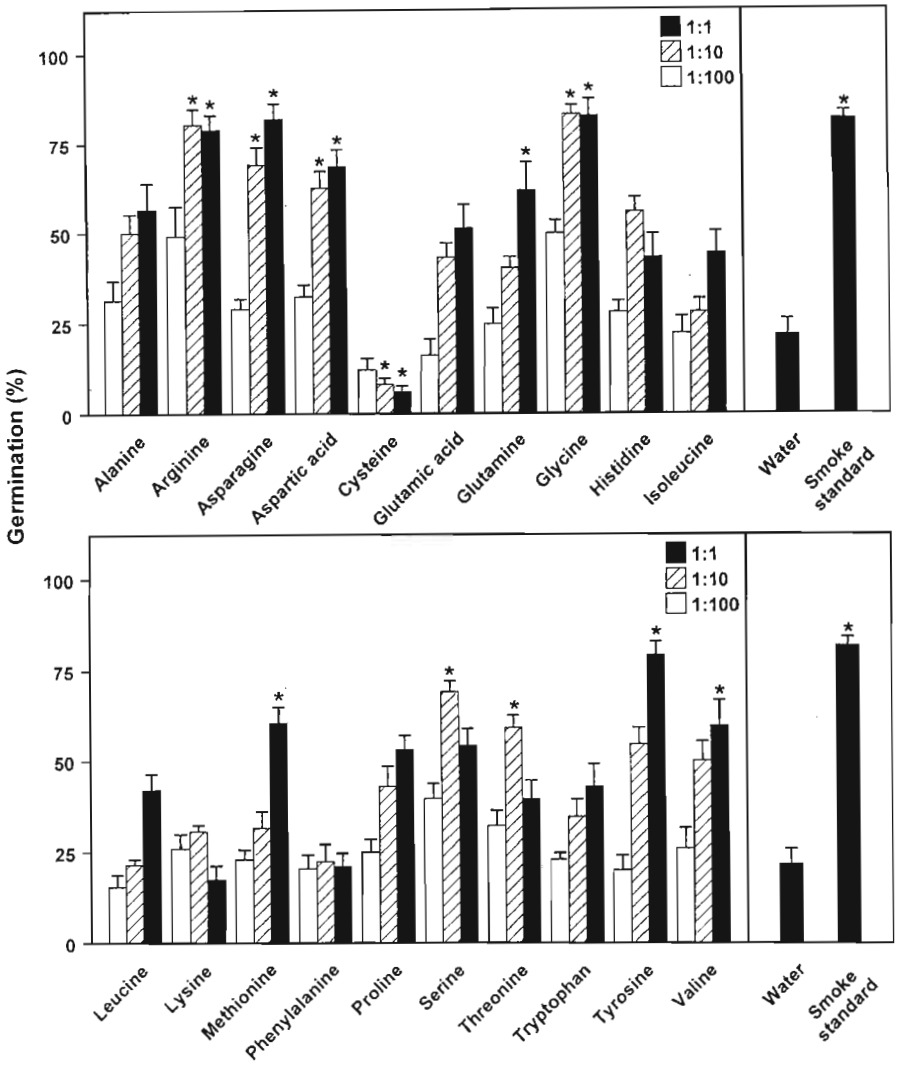

Figure 7.3: The effect of extracts, prepared from heating amino acids with glucose at 180 °C for 30 min, on the germination of Grand Rapids lettuce seeds after 24 h in the dark at 25 °C. Amino acids reacted with glucose in a molar ratio of 1:2, where 1 = 3.75 x 10^{-4} mol. Asterisks denote a significant ($p < 0.05$) difference from the water control; error bars indicate SE.

In particular, extracts prepared from arginine, asparagine, aspartic acid, glycine and tyrosine gave high levels of germination. In contrast, an extract prepared from cysteine and glucose resulted in germination levels below that observed with the water control (i.e., inhibition of germination).

These results indicate that the compound(s) responsible for germination stimulation can be formed from the amino-carbonyl reactions of a variety of amino-containing compounds with glucose. These "model systems", however, are known to form a highly complex mixture of reaction products (NURSTEN and O'REILLY, 1986). It is possible that some of these reaction products may have an inhibitory effect (as observed when cysteine was reacted with glucose).

As a result of the complex mixture of compounds formed during the reaction, the presence of compounds which promote germination may be counteracted for by the presence of inhibitory compounds. It is thus difficult to fully evaluate the quantitative amount of the germination promoter from the observed germination results, although the lettuce seed bioassay is useful for demonstrating the presence of the germination promoter.

7.3.3 Variation of the amino acid-to-glucose ratio

The results obtained with different amino acid and sugar ratios indicated that a higher proportion of glucose resulted in extracts which gave higher levels of germination (**Figure 7.4**). Extracts prepared from a 2:1 molar ratio (i.e. higher amino acid) generally gave lower levels of germination. Following these results, all further experiments were conducted using a 1:2 amino acid-to-sugar ratio.

7.3.4 Variation of reaction conditions

Reactions at different time and temperature combinations confirmed that the active products are formed between 160 and 220 °C (**Figure 7.5**). At 160 and 180 °C, a 5 min reaction time was insufficient to allow for production of the germination stimulant. The 5 min reaction at 160 °C did not result in the formation of the brown insoluble "foam", as observed for the other reaction conditions. The extracts from this reaction condition were much paler in comparison to extracts prepared at 160 °C for a longer reaction time, or for 5 min at the higher temperatures.

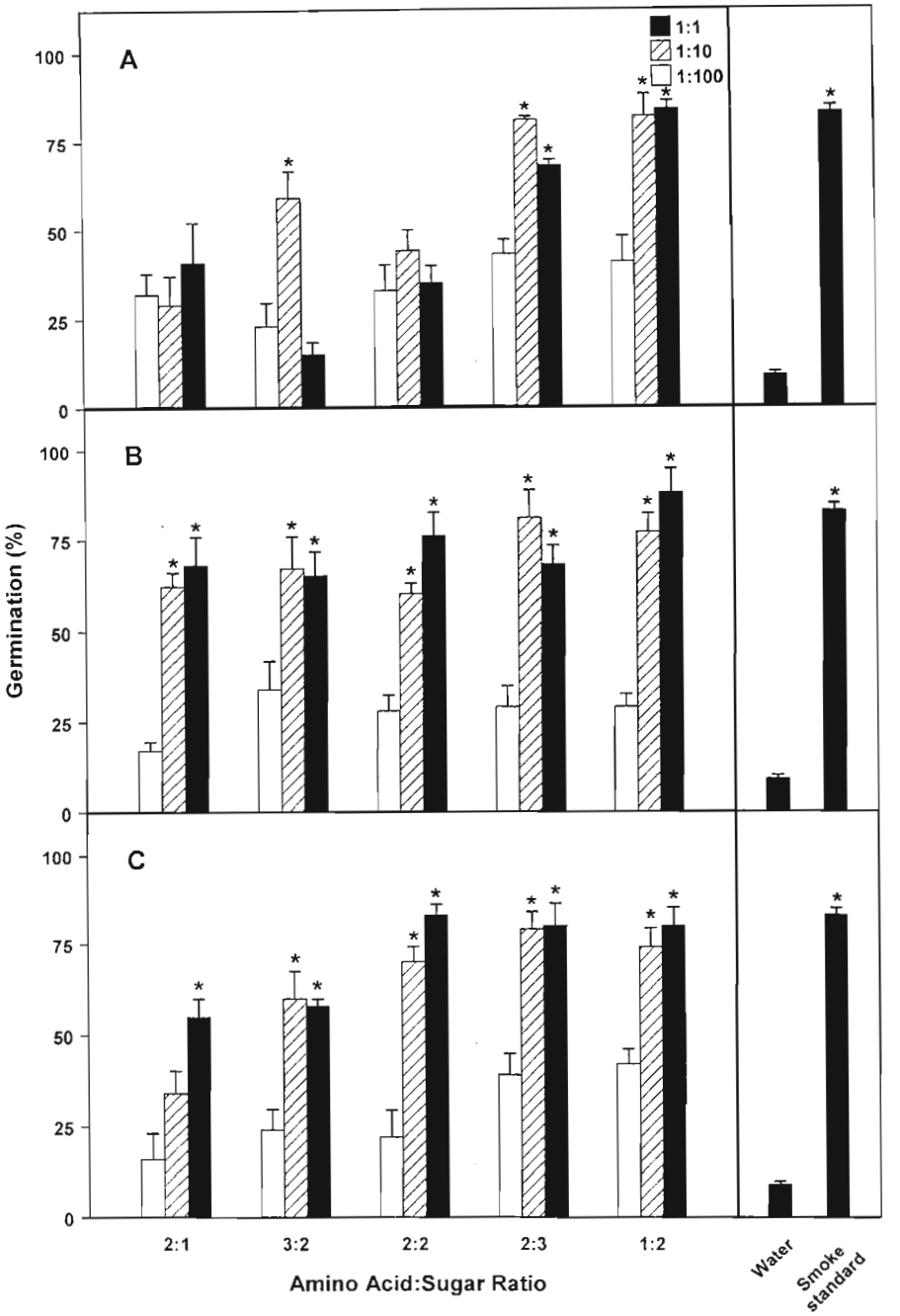

Figure 7.4: The effect of extracts, prepared from heating different ratios of (A) arginine, (B) asparagine, or (C) aspartic acid with glucose at 180 °C for 30 min, on the germination of Grand Rapids lettuce seeds after 24 h in the dark at 25 °C (1 = 3.75 x 10^{-4} mol). Asterisks denote a significant ($p < 0.05$) difference from the water control; error bars indicate SE.

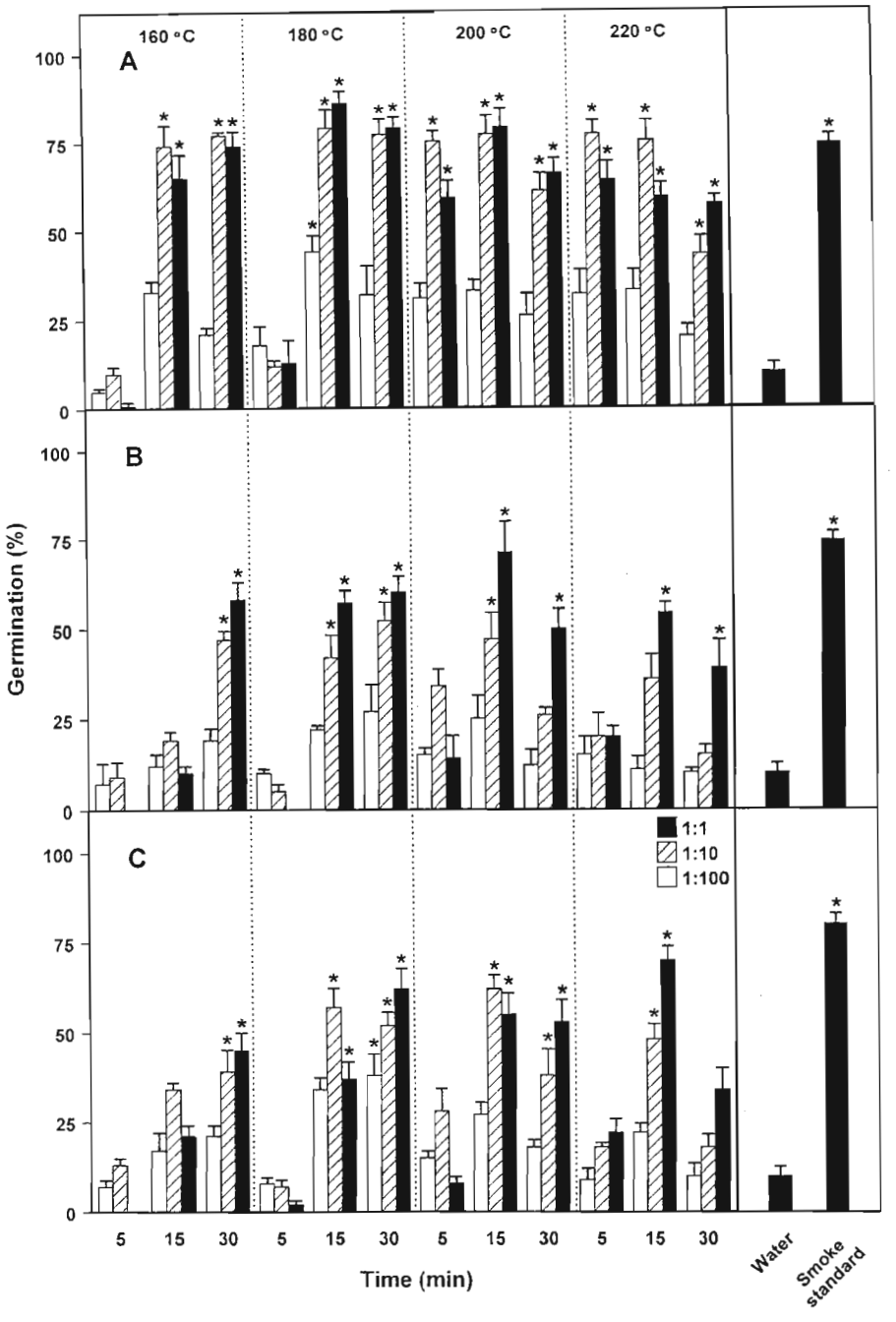

Figure 7.5: The effect of extracts, prepared from heating (A) arginine, (B) asparagine, or (C) aspartic acid with glucose at different time and temperature combinations, on the germination of Grand Rapids lettuce seeds after 24 h in the dark at 25 °C. Amino acids reacted with glucose in a molar ratio of 1:2, where 1 = 3.75 x 10^{-4} mol. Asterisks denote a significant ($p < 0.05$) difference from the water control; error bars indicate SE.

In general, however, an increase in reaction time, at each temperature, resulted in a decrease in colouration of the extract. As mentioned earlier, the colour of the extract did not appear to relate to the germination activity. At 220 °C, a longer reaction time (30 min) resulted in lower levels of germination in comparison to extracts obtained following a 15 min reaction. JÄGER, LIGHT and VAN STADEN (1996) observed a similar loss in germination activity from extracts prepared by heating dry leaves of *Themeda triandra* at this temperature. Subsequently, experiments were conducted at 180 °C for 30 min. However, for reactions in the custom-made apparatus, a higher reaction temperature of 220 °C was found to be more suitable, and volatilized compounds were collected in a water trap.

7.3.5 Reaction with different sugars

The results of reactions of arginine, asparagine and aspartic acid with different sugars are given in **Figure 7.6**. Results indicate that the active compound(s) can be formed from reactions between a number of amino acids and different sugars. All the sugars tested, in combination with these amino acids, gave a germination response, although the extracts prepared from D-ribose and D-xylose (both aldopentose sugars) gave the greatest response. Similar trends were observed with glycine, serine, tyrosine and valine. The combination of the tetrose sugar D-(-)-erythrose with glycine, however, showed no significant increase of germination above that of the water control.

Extracts were prepared from reactions of glycine or aspartic acid with D(-)mannitol or D(-)sorbitol. These combinations of amino acids and sugar alcohols as reactants did not result in the formation of the type of "brown foam" that was observed when amino acids were heated with sugars, and did not show improved germination above that of the water control.

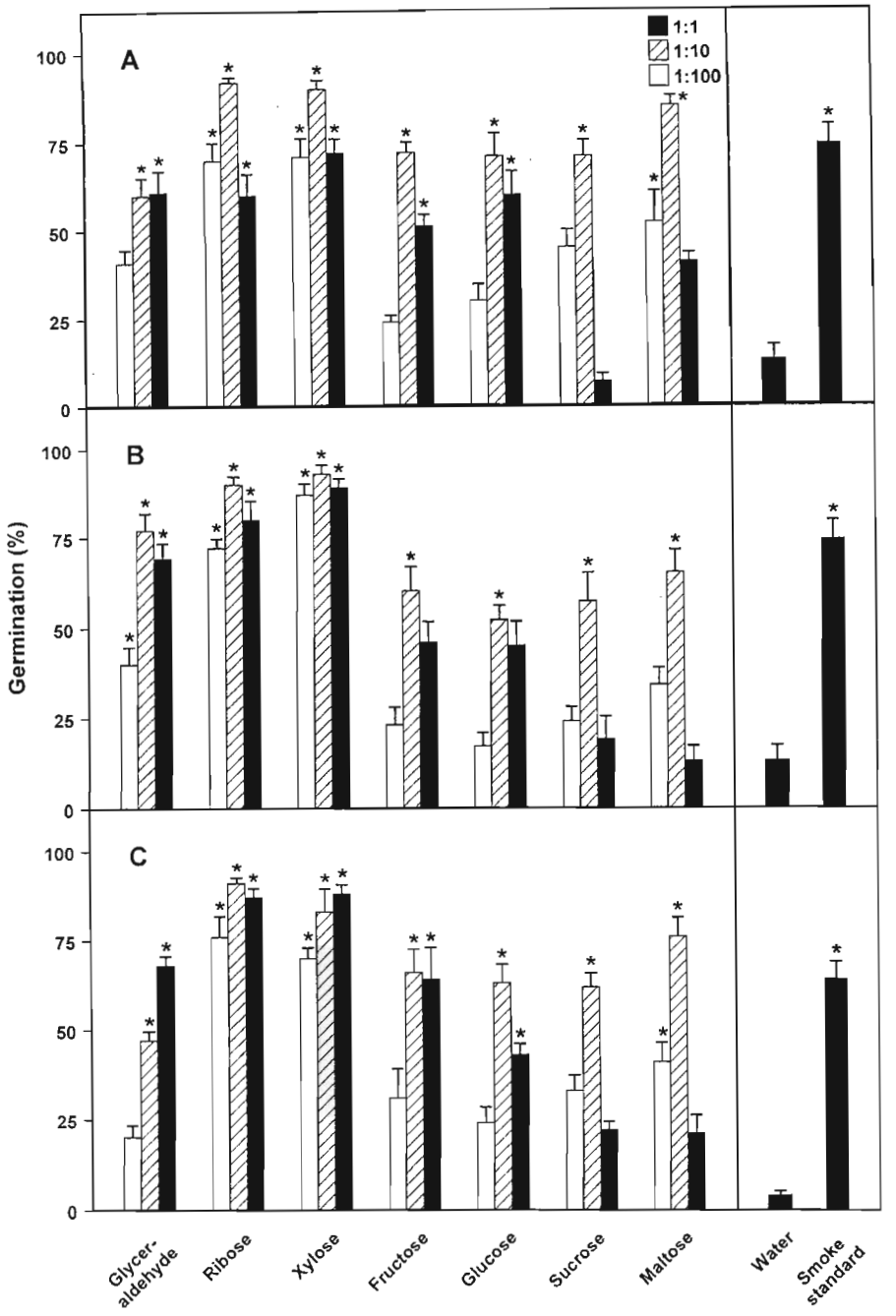

Figure 7.6: The effect of extracts, prepared from heating (A) arginine, (B) asparagine, or (C) aspartic acid with different sugars at 180 °C for 30 min, on the germination of Grand Rapids lettuce seeds after 24 h in the dark at 25 °C. Amino acids reacted with sugars in molar ratio of 1:2, where 1 = 3.75 x 10^{-4} mol. Asterisks denote a significant ($p < 0.05$) difference from the water control; error bars indicate SE.

7.3.6 HPLC and GC-MS analysis

The extracts prepared for HPLC analysis all exhibited germination activity in fractions eluting at 20-21 min. This is the same retention time at which the active fraction of the purified smoke solution eluted (**Section 6.2.1**; VAN STADEN, JÄGER, LIGHT and BURGER, 2004). In addition, the bulk extract prepared from heating glycine and xylose at 220 °C in the modified oven and purified using HPLC also showed germination activity at the same retention time (20-21 min) as the active compound isolated from the plant-derived smoke solution (**Section 6.2.1**; VAN STADEN, JÄGER, LIGHT and BURGER, 2004).

Although in the HPLC analysis this fraction appeared to contain only one pure active principle, GC-MS analysis, in which a universal and highly sensitive detection device is used, gave a total ion chromatogram (TIC) that revealed the presence of a large number of constituents. A sample of the germination cue from plant-derived smoke, 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one (**Section 6.2.1**; VAN STADEN, JÄGER, LIGHT and BURGER, 2004), was available for GC-MS retention-time comparison. Locating the active principle in the synthetic product under investigation was further simplified by plotting reconstructed single ion chromatograms for the two most abundant ions, *m/z* 121 and 150, in the spectrum of the plant-derived germination cue.

The compound eluting at the same retention time as the plant-derived active principle has a mass spectrum (**Figure 7.7A**) containing the diagnostic ions, *m/z* 150 (65); 149 (9); 122 (20); 121 (100); 105 (3); 94 (9); 93 (7); 75 (3); 74(3); 66 (18); 65 (26); 51 (8); 50 (7); 39 (35); 38 (8); 29 (8); 27 (3%).

Critical evaluation of the results of the present investigation against the background of our previous results (**Chapter 6**; VAN STADEN, JÄGER, LIGHT and BURGER, 2004) revealed that the germination stimulant isolated from plant-derived smoke and the germination stimulant prepared from glycine and xylose elute at the same elution volume in HPLC, elute at the same retention time in GC when co-injected, and have identical mass spectra (**Figure 7.7**). This clearly shows that the compound prepared

by heating a mixture of xylose and glycine is identical to the recently identified germination cue, 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one. Furthermore, it shows that the germination stimulant in plant-derived smoke can also be produced by heating mixtures of certain amino acids and sugars.

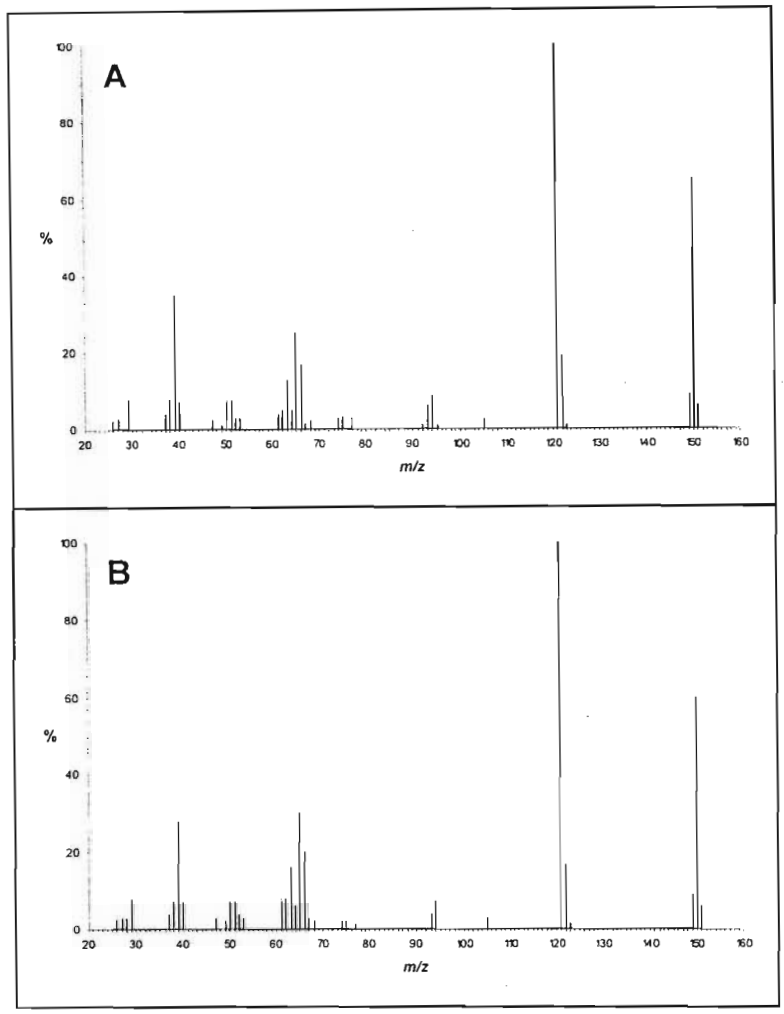

Figure 7.7: Mass spectra of (A) a constituent extracted from the product formed by heating glycine and xylose, and (B) the germination promoter, 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one, isolated from plant-derived smoke.

It is interesting to note that hemicelluloses, which are abundant in both primary and secondary cell walls of plants, are polymers of sugars such as arabinose, mannose, and xylose, the pentose sugar, which showed a good germination response when heated in combination with certain amino acids. The germination effect observed with heating cellulose and hemicelluloses (KEELEY and PIZZORNO, 1986; JÄGER, LIGHT and

VAN STADEN, 1996) may possibly be attributed to trace impurities in the starting materials.

During a wildfire, the soil, which contains some organic matter, would also be heated to high temperatures, possibly resulting in the production of germination-stimulating compounds. Soil temperatures during fires depend on the depth and type of fuel but can be as high as 600 ˚C at the surface and between 50 and 225 ˚C at depths of 2 cm (MARTIN, 1966; BRADSTOCK, AULD, ELLIS and COHN, 1992). Extracts from soil heated at 195 ˚C for 10 min were shown to stimulate the germination of seeds of two chaparral herbs (KEELEY and NITZBERG, 1984), and extracts from heated soils also increased the emergence of common plant species of the sagebrush-steppe of the western United States (BLANK and YOUNG, 1998).

The chemistry of Maillard reactions and pyrolysis reactions is certainly extremely complex. Although 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one has not been previously identified in Maillard reaction products, it is of interest that we have observed germination stimulation in the lettuce seed bioassay with extracts of instant coffee and toasted bread ("roasted" food products). The results presented in these experiments provide further information on the germination cue found in smoke that is produced during wildfires from ubiquitously occurring organic compounds.

7.4 SUMMARY

- Heating proteins and glucose at 180 °C for 30 min formed water-soluble reaction products which promoted germination in the lettuce seed bioassay
- Heating certain amino acids and glucose at 180 °C for 30 min formed water-soluble reaction products which promoted germination in the lettuce seed bioassay
- Reactions which used a higher proportion of glucose (to amino acid) resulted in greater germination in the lettuce seed bioassay
- Heating certain amino acids with different sugars at 180 °C for 30 min formed water-soluble reaction products which promoted germination in the lettuce seed bioassay
- Reactions of certain amino acids with ribose or xylose gave the greatest response in the lettuce seed bioassay
- HPLC fractionation of extracts from reactions between certain amino acids and sugars gave germination activity in fractions eluting at 20-21 min (the same elution time as the germination cue from plant-derived smoke)
- Further analysis, using GC-MS, of an extract from a reaction between glycine and xylose, revealed the presence of 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one in the active fraction
- This compound has not previously been identified as a product from Maillard reactions
- The principal germination cue found in plant-derived smoke is formed from ubiquitously occurring organic compounds

7.5 COLLABORATION

The GC-MS experimental work was conducted by Prof. B.V. Burger (Laboratory for Ecological Chemistry, Department of Chemistry, University of Stellenbosch).

8 General Conclusions

And suppose we solve all the problems it presents?
What happens? We end up with more problems than we started with.
Because that's the way problems propagate their species.
A problem left to itself dries up or goes rotten. But fertilize a problem with a solution - you'll hatch out dozens.

~ N.F. Simpson ~
A Resounding Tinkle

During the course of completing this thesis, our research group was able to identify the major germination cue from plant-derived smoke, 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one (VAN STADEN, JÄGER, LIGHT and BURGER, 2004). This novel compound was also isolated and identified from burnt cellulose by FLEMATTI, GHISALBERTI, DIXON and TRENGOVE (2004), researchers from Western Australia. This discovery is an enormous breakthrough in the field and now opens a way forward to a greater understanding of the physiological processes which are involved in smoke-stimulated seed germination. Furthermore, the use of this compound as a germination cue holds great potential for a wide variety of applications in agriculture, horticulture, forestry, conservation, ecosystem rehabilitation and weed science.

In a recent article by LEVY (2005), Keeley makes the following comment: *"Many California chaparral plants will sprout only in the first year following a fire. Seeds then go dormant and don't sprout until another blaze passes through. Yet Dixon and his colleagues have shown that butenolide can work at tiny doses - in the order of parts per trillion. Those levels of butenolide are likely to remain in soils for years after a fire. If butenolide is the key, how do those plants maintain dormancy?"* This is a good point that Keeley makes, and one for which there is currently no satisfactory answer. It has been observed that the germination cue from smoke can be retained in the soil well beyond the time of burning, or of smoke solution application (ROCHE, DIXON and PATE, 1998; PRESTON and BALDWIN, 1999). Interestingly though, KEELEY and NITZBERG (1984) demonstrated that sterile extracts made from heated soil resulted in

a comparable germination response to that obtained with the heated soil itself. However, if the soil was moistened and incubated prior to the preparation of the sterile extract, a significant reduction in germination was observed. It is possible that the growth of soil microorganisms may result in toxins that counteract the effect of the water-soluble germination compound, or alternatively the compound may be metabolized by soil microorganisms. Certainly, the longevity of the germination cue in soils is one aspect that will require further research, particularly with respect to the use of 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one in agricultural systems.

We have recently tested 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one for possible mutagenic and genotoxic effects using the VITOTOX® test and the Ames assay (VERSCHAEVE, MAES, LIGHT and VAN STADEN, 2006). Importantly, the results indicated that the compound is not toxic nor genotoxic, at the levels tested.

The biggest question remaining is that of the mode of action of 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one in promoting germination. The possibility of now conducting in-depth physiological studies using a single active compound, instead of a crude smoke solution containing thousands of compounds, is an exciting step forward. Furthermore, plant molecular biology can be used a tool for unravelling some of the underlying mechanisms and genetic controls which may be involved.

The experiments presented in this thesis collectively provide some answers to some of the physiological aspects of smoke as a germination cue. However, as is the case most often in science, in trying to find answers for certain questions, one inevitably creates even more questions.

9 References

ABELES, F.B., LONSKI, J. (1969). Stimulation of lettuce seed germination by ethylene. *Plant Physiology* 44:277-280.

ACEA, M.J., CARBALLAS, T. (1996). Changes in physiological groups of microorganisms in soil following wildfire. *FEMS Microbiology Ecology* 20:33-39.

ADKINS, S.W., DAVIDSON, P.J., MATTHEW, L., NAVIE, S.C., WILLS, D.A., TAYLOR, I.N., BELLAIRS, S.M. (2000). Smoke and germination of arable and rangeland weeds. In: *Seed Biology: Advances and Applications*. Eds: Black, M., Bradford, K.J., Vázquez-Ramos, J. CABI Publishing. ISBN 0-85199-404-0. pp 347-359.

ADKINS, S.W., PETERS, N.C.B. (2001). Smoke derived from burnt vegetation stimulates germination of arable weeds. *Seed Science Research* 11:213-222.

ADKINS, S.W., PETERS, N.C.B., PATERSON, M.F., NAVIE, S.C. (2003). Germination stimulation of weed species by smoke. In: *The Biology of Seeds: Recent Research Advances*. Eds: Nicolás, G., Bradford, K.J., Côme, D., Pritchard, H.W. CABI Publishing. ISBN 0-85199-653-1. pp 413-420.

ADRIANSZ, T.D., RUMMEY, J.M., BENNET, I.J. (2000). Solid phase extraction and subsequent identification by gas-chromatography-mass-spectrometry of a germination cue present in smoky water. *Analytical Letters* 33:2793-2804.

AFOLAYAN, A.J., MEYER, J.J.M., LEEUWNER, D.V. (1997). Germination in *Helichrysum aureonitens* (Asteraceae): Effects of temperature, light, gibberellic acid, scarification and smoke extract. *South African Journal of Botany* 63:22-24.

ALLAN, S.M., ADKINS, S.W., PRESTON, C.A., BELLAIRS, S.M. (2004). Improved germination of the Australian natives: *Hibbertia commutata*, *Hibbertia amplexicaulis* (Dilleniaceae), *Chameascilla corymbosa* (Liliaceae) and *Leucopogon nutans* (Epacridaceae). *Australian Journal of Botany* 52:345-351. Corrigendum, 52:559.

ANDERSSON, M., MICHELSEN, A., JENSEN, M., KJØLLER, A. (2004). Tropical savannah woodland: effects of experimental fire on soil microorganisms and soil emissions of carbon dioxide. *Soil Biology & Biochemistry* 36:849-858.

ANDREOLI, C., GIGANTE, D., NUNZIATA, A. (2003). A review of in vitro methods to assess the biological activity of tobacco smoke with the aim of reducing the toxicity of smoke. *Toxicology in Vitro* 17:587-594.

ARCHIBOLD, O.W. (1995). *Ecology of World Vegetation*. Chapman & Hall, London. ISBN 0-412-44290-6. pp 1-510.

ASITA, A.O., CAMPBELL, I.A. (1990). Anti-microbial activity of smoke from different woods. *Letters in Applied Microbiology* 10:93-95.

AULD, T.D., BRADSTOCK, R.A. (1996). Soil temperatures after the passage of a fire: Do they influence the germination of buried seeds? *Australian Journal of Ecology* 21:106-109.

BAKER, K.S., STEADMAN, K.J., PLUMMER, J.A., MERRITT, DIXON, K.W. (2005a). The changing window of conditions that promotes germination of two fire ephemerals, *Actinotus leucocephalus* (Apiaceae) and *Tersonia cyathiflora* (Gyrostemonaceae). *Annals of Botany* 96:1225-1236.

BAKER, K.S., STEADMAN, K.J., PLUMMER, J.A., MERRITT, D.J., DIXON, K.W. (2005b). Dormancy release in Australian fire ephemeral seeds during burial increases germination response to smoke water or heat. *Seed Science Research* 15:339-348.

BALDWIN, I.T., MORSE, L. (1994). Up in smoke. II. Germination of *Nicotiana attenuata* in response to smoke-derived cues and nutrients in burned and unburned soils. *Journal of Chemical Ecology* 20:2373-2391.

BALDWIN, I.T., STASZAK-KOZINSKI, L., DAVIDSON, R. (1994). Up in smoke. I. Smoke-derived germination cues for post-fire annual, *Nicotiana attenuata* Torr ex Watson. *Journal of Chemical Ecology* 20:2345-2371.

BALTES, W., BOCHMANN, G. (1986). Model reaction on roast aroma formation - The reaction of serine and threonine with sucrose. In: *Amino-carbonyl Reactions in Food and Biological Systems.* Eds: Fujimaki, M., Namiki, M., Hiromichi, K. Elsevier Science Publishers, Amsterdam. ISBN 0-444-99510-2. pp 245-255.

BASKIN, C.C., BASKIN, J.M. (1998). *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination.* Academic Press, San Diego. ISBN 0-12-080260-0. pp 1-666.

BAXTER, B.J.M., GRANGER, J.E., VAN STADEN, J. (1995). Plant-derived smoke and seed germination: is all smoke good smoke? That is the burning question. *South African Journal of Botany* 61:275-277.

BAXTER, B.J.M., VAN STADEN, J. (1994). Plant-derived smoke: an effective seed pre-treatment. *Plant Growth Regulation* 14:279-282.

BAXTER, B.J.M., VAN STADEN, J., GRANGER, J.E., BROWN, N.A.C. (1994). Plant-derived smoke and smoke extracts stimulate seed germination of the fire-climax grass *Themeda triandra* Forssk. *Environmental and Experimental Botany* 34:217-223.

BEKAARDT, C.R., PIETERSE, P.J., COETZEE, J.H., AGENBAG, G.A. (2004). The breaking of seed dormancy of guayule (*Parthenium argentatum* Gray.) by treatment with gibberellic acid and smoke water solution. *South African Journal of Plant Soil* 21:101-103.

BELIGNI, M.V., LAMATTINA, L. (2000). Nitric oxide stimulates seed germination and de-etiolation, and inhibits hypocotyl elongation, three light-inducible responses in plants. *Planta* 210:215-221.

BELL, D.T., VLAHOS, S., WATSON, L.E. (1987). Stimulation of seed germination of understorey species of the northern jarrah forest of Western Australia. *Australian Journal of Botany* 35:593-599.

BELL, D.T. (1999). Turner Review No. 1 - The process of germination in Australian species. *Australian Journal of Botany* 47:475-517.

BELL, D.T., KING, L.A., PLUMMER, J.A. (1999). Ecophysiological effects of light quality and nitrate on seed germination in species from Western Australia. *Australian Journal of Ecology* 24:2-10.

BELLAIRS, S.M., BARTIER, F.V., GRAVINA, A.J., BAKER, K. (2006). Seed biology implications for the maintenance and establishment of *Tetratheca juncea* (Tremandraceae), a vulnerable Australian species. *Australian Journal of Botany* 54:35-41.

BENECH-ARNOLD, R.L., SÁNCHEZ, R.A., FORCELLA, R., KRUK, B.C., GHERSA, C.M. (2000). Environmental control of dormancy in weed seed banks in soil. *Field Crops Research* 67:105-122.

BERRIE, A.M.M. (1966). The effect of temperature and light on the germination of lettuce seeds. *Physiologia Plantarum* 19:429-436.

BERRIE, A.M.M., HENDRIE, M.R., PARKER, W., KNIGHTS, B.A. (1967). Induction of light sensitive dormancy in seed of *Lactuca sativa* L. (lettuce) by patulin. *Plant Physiology* 42:889-890.

BERRIE, A.M.M., ROBERTSON, J. (1976). Abscisic acid as an endogenous component in lettuce fruits, *Lactuca sativa* L. cv. Grand Rapids. Does it control thermodormancy? *Planta* 131:211-215.

BETHKE, P.C., GUBLER, F., JACOBSEN, J.V., JONES, R.L. (2004). Dormancy of *Arabidopsis* seeds and barley grains can be broken by nitric oxide. *Planta* 219:847-855.

BEWLEY, J.D., NEGBI, M., BLACK, M. (1968). Immediate phytochrome action in lettuce seeds and its interaction with gibberellins and other germination promoters. *Planta* 78:351-357.

BEWLEY, J.D., FOUNTAIN, D.W. (1972). A distinction between the actions of abscisic acid, gibberellic acid and cytokinins in light-sensitive lettuce seed. *Planta* 102:368-371.

BHATIA, N.P., NKANG, A.E., WALSH, K.B., BAKER, A.J.M., ASHWATH, N., MIDMORE, D.J. (2005). Successful seed germination of the nickel hyperaccumulator *Stackhousia tryonii. Annals of Botany* 96:159-163.

BLANK, R.R., YOUNG, J.A. (1998). Heated substrate and smoke: Influence on seed emergence and plant growth. *Journal of Range Management* 51:577-583.

BLOMMAERT, K.L.J. (1972). Buchu seed germination. *Journal of South African Botany* 38:237-239.

BOND, P., GOLDBLATT, P. (1984). Plants of the Cape flora. A descriptive catalogue. *Journal of South African Botany* 13(supplement):9.

BOND, W.J., VLOK, J., VIVIERS, M. (1984). Variation in seedling recruitment of Cape Proteaceae after fire. *Journal of Ecology* 72:209-221.

BOND, W.J. (1985). Canopy-stored seed reserves (serotiny) in Cape Proteaceae. *South African Journal of Botany* 51:181-186.

BOND, W.J., VAN WILGEN, B.W. (1996). *Fire and Plants*. Chapman & Hall, London, UK. ISBN 0-412-47540-5. pp 1-263.

BORTHWICK, H.A., HENDRICKS, S.B., PARKER, M.W., TOOLE, E.H., TOOLE, V.K. (1952). A reversible photoreaction controlling seed germination. *Proceedings of the National Academy of Sciences of the USA* 38:662-666.

BORTHWICK, H.A., HENDRICKS, S.B., TOOLE, E.H., TOOLE, V.K. (1954). Action of light on lettuce-seed germination. *Botanical Gazette* 115:205-225.

BOUCHER, C. (1981). Autecological and population studies of *Orothamnus zeyheri* in the Cape of South Africa. In: *The Biological Aspects of Rare Plant Conservation*. Ed: Synge, H. John Wiley & Sons, Chichester. ISBN 0-471-28004-6. pp 343-353.

BOUCHER, C., MEETS, M. (2004). Determination of the relative activity of aqueous plant-derived smoke solutions used in seed germination. *South African Journal of Botany* 70:313-318.

BRADSTOCK, R.A., AULD, T.D., ELLIS, M.E., COHN, J.S. (1992). Soil temperatures during bushfires in semi-arid, mallee shrublands. *Australian Journal of Ecology* 17:433-440.

BRAUN, J.W., KHAN, A.A. (1975). Endogenous abscisic acid levels in germinating and nongerminating lettuce seed. *Plant Physiology* 56:731-733.

BRINGMANN, G., NOLL, T., RISCHER, H. (2002). *In vitro* germination and establishment of tissue cultures of *Bulbine caulescens* and of two *Kniphofia* species (Asphodelaceae). *Plant Cell Reports* 21:125-129.

BRITS, G.J. (1986). Influence of fluctuating temperatures and H_2O_2 treatment on germination of *Leucospermum cordifolium* and *Serruria florida* (Proteaceae) seeds. *South African Journal of Botany* 52:286-290.

BROWN, N.A.C. (1993a). Promotion of germination of fynbos seeds by plant-derived smoke. *New Phytologist* 123:575-583.

BROWN, N.A.C. (1993b). Seed germination in the fynbos fire ephemeral, *Syncarpha vestita* (L.) B. Nord. is promoted by smoke, aqueous extracts of smoke and charred wood derived from burning the ericoid-leaved shrub, *Passerina vulgaris* Thoday. *International Journal of Wildland Fire* 3:203-206.

BROWN, N.A.C., BOTHA, P.A., KOTZE, G., JAMIESON, H. (1993). Where there's smoke there's seed. *Veld and Flora* 79:77-79.

BROWN, N.A.C., KOTZE, G., BOTHA, P.A. (1993). The promotion of seed germination of Cape *Erica* species by plant-derived smoke. *Seed Science and Technology* 21:179-185.

BROWN, N.A.C. (1994). First the gas, now instant dehydrated smoke. *Veld and Flora* 80:72-73.

BROWN, N.A.C., JAMIESON, H., BOTHA, P.A. (1994). Stimulation of seed germination in South African species of Restionaceae by plant-derived smoke. *Plant Growth Regulation* 15:93-100.

BROWN, N.A.C., BOTHA, P.A. (1995). List of species in which treatment with smoke or aqueous smoke extract has been shown to give improved germination. *Veld and Flora*. 81:93.

BROWN, N.A.C., VAN STADEN, J. (1997). Smoke as a germination cue: A review. *Plant Growth Regulation* 22:115-124.

BROWN, N.A.C., PROSCH, D.S., BOTHA, P.A. (1998). Plant derived smoke: An effective pre-treatment for seeds of *Syncarpha* and *Rhodocoma* and potentially for many other Fynbos species. *South African Journal of Botany* 64:90-92.

BROWN, N.A.C., VAN STADEN, J. (1998). Plant-derived smoke: An effective seed pre-soaking treatment for wildflower species and with potential for horticultural and vegetable crops. *Seed Science and Technology* 26:669-673.

BROWN, N.A.C. (1999). The role of fire in enhancing regeneration: The Cape Floral Region. In: *A Colour Atlas of Plant Propagation and Conservation.* Ed: Bowes, B.G. Manson Publishing Ltd, London. ISBN 1-874545-92-8. pp 157-167.

BROWN, N., BOTHA, P. (2002). Smoking seeds. An updated list of fynbos species with seeds that have a good germination response to smoke. *Veld and Flora* 88:68-69.

BROWN, N.A.C., VAN STADEN, J., DAWS, M.I., JOHNSON, T. (2003). Patterns in the seed germination response to smoke in plants from the Cape Floristic Region, South Africa. *South African Journal of Botany* 69:514-525.

BROWN, N.A.C., BOTHA, P.A. (2004). Smoke seed germination studies and a guide to seed propagation of plants from the major families of the Cape Floristic Region, South Africa. *South African Journal of Botany* 70:559-581.

BURDETT, A.N., VIDAVER, W.E. (1971). Synergistic action of ethylene with gibberellin or red light in germinating lettuce seeds. *Plant Physiology* 48:656-657.

CARPITA, N.C., ROSS, C.W., NABORS, M.W. (1979). The influence of plant growth regulators on the growth of the embryonic axes of red- and far-red-treated lettuce seeds. *Planta* 145:511-516.

CASAL, J.J., SÁNCHEZ, R.A. (1998). Phytochromes and seed germination. *Seed Science Research* 8:317-329.

CHENG, F.-Y., HSU, S.-Y., KAO, C.H. (2002). Nitric oxide counteracts the senescence of detached rice leaves induced by dehydration and polyethylene glycol but not by sorbitol. *Plant Growth Regulation* 38:265-272.

CHIWOCHA,S.D.S., ABRAMS,S.R., AMBROSE, S.J., CUTLER, A.J., LOEWEN, M., ROSS, A.R.S., KERMODE, A.R. (2003). A method for profiling classes of plant hormones ad their metabolites using liquid chromatography-electrospray ionization tandem mass spectrometry: an analysis of hormone regulation of thermodormancy of lettuce (*Lactuca sativa* L.) seeds. *The Plant Journal* 35:405-417.

CHRISTENSEN, N.L. (1973). Fire and the nitrogen cycle in California chaparral. *Science* 181:66-68.

CHRISTENSEN, N.L., MULLER, C.H. (1975). Effects of fire on factors controlling plant growth in *Adenostoma* chaparral. *Ecological Monographs* 45:29-45.

CLARKE, P.J., DAVISON, E.A., FULLOON, L. (2000). Germination and dormancy of grassy woodland and forest species: effects of smoke, heat, darkness and cold. *Australian Journal of Botany* 48:687-700.

CLARKE, S., FRENCH, K. (2005). Germination response to heat and smoke of 22 Poaceae species from grassy woodlands. *Australian Journal of Botany* 53:445-454.

CLOSE, D.C., WILSON, S.J. (2002). Provenance effects on pre-germination treatments for *Eucalyptus regnans* and *E. delegatensis* seed. *Forest Ecology and Management* 170:299-305.

COOK, C.E., WHICHARD, L.P., WALL, M.E., EGLEY, G.H., COGGON, P., LUHAN, P.A., McPHAIL, A.T. (1972). Germination stimulants. II. The structure of strigol - a potent seed germination stimulant for witchweed (*Striga lutea* Lour.). *Journal of the American Chemical Society* 94:6198-6199.

CROSTI, R., LADD, P.G., DIXON, K.W., PIOTTO, B. (2006). Post-fire germination: The effect of smoke on seeds of selected species from the central Mediterranean basin. *Forest Ecology and Management* 221:306-312.

DALLMAN, P.R. (1998). *Plant Life in the World's Mediterranean Climates.* Oxford University Press, Oxford. ISBN 0-19-850464-0. pp 1-257.

DE LANGE, J.H., BOUCHER, C. (1990). Autecological studies on *Audouinia capitata* (Bruniaceae). I. Plant-derived smoke as a seed germination cue. *South African Journal of Botany* 56:700-703.

DE LANGE, J.H., BOUCHER, C. (1993a). Autecological studies on *Audouinia capitata* (Bruniaceae). 4. Seed production, seed banks and seedling recruitment. *South African Journal of Botany* 59:145-155.

DE LANGE, J.H., BOUCHER, C. (1993b). Autecological studies on *Audouinia capitata* (Bruniaceae). 8. Role of fire in regeneration. *South African Journal of Botany* 59:188-202.

DEBANO, L.F., CONRAD, C.E. (1978). The effect of fire on nutrients in a chaparral ecosystem. *Ecology* 59:489-497.

DERKX, M.P.M., VERMEER, E., KARSSEN, C.M. (1994). Gibberellins in seeds of *Arabidopsis thaliana*. Biological activities, identification and effects of light and chilling on endogenous levels. *Plant Growth Regulation* 15:223-234.

DI CASTRI, F. (1981). Mediterranean-type shrublands of the world. In: *Ecosystems of the World: 11. Mediterranean-type Shrublands.* Eds: di Castri, F., Goodall, D.W., Specht, R.L. Elsevier Scientific Publishing Company, Amsterdam. ISBN 0-444-41858-X. pp 1-52.

DIAZ-FIERROS, F., BENITO, E., VEGA, J.A., CASTELAO, A., SOTO, B, PÉREZ, R., TABOADA, T. (1990). Solute loss and soil erosion in burnt soil from Galicia (NW Spain). In: *Fire in Ecosystem Dynamics.* Eds: Goldammer, J.G., Jenkins, M.J. SPB Academic Publishing bv, The Hague, The Netherlands. ISBN 90-5103-045-2. pp 103-116.

DIXON, K.W., ROCHE, S. (1995). The role of combustion products (smoke) in stimulating *ex-situ* and *in-situ* germination of Western Australian plants. *Combined Proceedings of the International Plant Propagator's Society* 45:53-56.

DIXON, K.W., ROCHE, S., PATE, J.S. (1995). The promotive effect of smoke derived from burnt native vegetation on seed germination of Western Australian plants. *Oecologia* 101:185-192.

DIXON, K., BARRETT, R. (2003). Defining the role of fire in south-west Western Australian plants. In: *Fire in ecosystems of south-west Western Australia: impacts and management.* Eds: Abbott, I., Burrows, N. Backhuys Publishers, Leiden, The Netherlands. ISBN 90-5782-131-1. pp 205-223.

DOHERTY, L.C., COHN, M.A. (2000). Seed dormancy in red rice (*Oryza sativa*). XI. Commercial liquid smoke elicits germination. *Seed Science Research* 10:415-421.

DREWES, F.E., SMITH, M.T., VAN STADEN, J. (1995). The effect of a plant-derived smoke extract on the germination of light-sensitive lettuce seed. *Plant Growth Regulation* 16:205-209.

DU TOIT, S.R., CAMPBELL, E.E. (1999). The effect of fire on two Eastern Cape *Cyclopia* species (Fabaceae). *South African Journal of Botany* 65:203-207.

DURNER, J., KLESSIG, S.F. (1999). Nitric oxide as a signal in plants. *Current Opinion in Plant Biology* 2:369-374.

EGERTON-WARBURTON, L.M. (1998). A smoke-induced alteration of the sub-testa cuticle in seeds of the post-fire recruiter, *Emmenanthe penduliflora* Benth. (Hydrophyllaceae). *Journal of Experimental Botany* 49:1317-1327.

ENRIGHT, N.J., GOLDBLUM, D., ATA, P., ASHTON, D.H. (1997). The independent effects of heat, smoke and ash on emergence of seedlings from the soil seed bank of a healthy *Eucalyptus* woodland in Grampians (Gariwerd) National Park, western Victoria. *Australian Journal of Ecology* 22:81-88.

ENRIGHT, N.J., KINTRUP, A. (2001). Effects of smoke, heat and charred wood on the germination of dormant soil-stored seeds from a *Eucalyptus baxteri* heathy-woodland in Victoria, SE Australia. *Austral Ecology* 26:132-141.

ERDMAN, A.M., WATTS, B.M., ELLIAS, L.C. (1954). Smoke flavor and ascorbic acid as preservatives for fatty fish. *Food Technology* 8:320-323.

ERIKSSON, C.E.A., ASP, N.-G., THEANDER, O. (1981). Preface. In: *Maillard Reactions in Food: Chemical, Physiological and Technological Aspects.* Ed: Eriksson, C. Pergamon Press, Oxford. ISBN 0-08-025496-9. pp 1-3.

FLEMATTI, G.R., GHISALBERTI, E.L., DIXON, K.W., TRENGOVE, R.D. (2001). A re-evaluation of cineole as a germination promoter of *Lactuca sativa* L. Grand Rapids. *Analytical Letters* 34:2221-2225.

FLEMATTI, G.R., GHISALBERTI, E.L., DIXON, K.W., TRENGOVE, R.D. (2004). A compound from smoke that promotes seed germination. *Science* 305:977.

FLEMATTI, G.R., GHISALBERTI, E.L., DIXON, K.W., TRENGOVE, R.D. (2005). Synthesis of the seed germination stimulant 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one. *Tetrahedron Letters* 46:5719-5721.

GARDNER, G. (1983). The effect of growth retardants on phytochrome-induced lettuce seed germination. *Journal of Plant Growth Regulation* 2:159-163.

GARDNER, M.J., DALLING, K.J., LIGHT, M.E., JÄGER, A.K., VAN STADEN, J. (2001). Does smoke substitute for red light in the germination of light-sensitive lettuce seeds by affecting gibberellin metabolism? *South African Journal of Botany* 67:636-640.

GIBA, Z., GRUBIŠIĆ, D., TODOROVIĆ, S., SAJC, L., STOJAKOVIĆ, Đ., KONJEVIĆ, R. (1998). Effect of nitric oxide releasing compounds on phytochrome-controlled germination of Empress tree seeds. *Plant Growth Regulation* 26:175-181.

GILMOUR, C.A., CROWDEN, R.K., KOUTOULIS, A. (2000). Heat shock, smoke and darkness: partner cues in promoting seed germination in *Epacris tasmanica* (Epacridaceae). *Australian Journal of Botany* 48:603-609.

GOLDBLATT, P. (1978). An analysis of the flora of Southern Africa: its characteristics, relationships, and origins. *Annals of the Missouri Botanical Gardens* 65:369-436.

GOMAA, E.A., GRAY, J.I., RABIE, S., LOPEZ-BOTE, C., BOOREN, A.M. (1993). Polycyclic aromatic hydrocarbons in smoked food products and commercial liquid smoke flavouring. *Food Additives and Contaminants* 10:503-521.

GONAI, T., KAWAHARA, S., TOUGOU, M., SATOH, S., HASHIBA, T., HIRAI, N., KAWAIDE, H., KAMIYA, Y., YOSHIOKA, T. (2004). Abscisic acid in the thermoinhibition of lettuce seed germination and enhancement of its catabolism by gibberellin. *Journal of Experimental Botany* 55:111-118.

GONZÁLEZ-RABANAL, F., CASAL, M. (1995). Effect of high temperatures and ash on germination of ten species from gorse shrubland. *Vegetatio* 116:123-131.

GOUDEY, J.S., SAINI, H.S., SPENCER, M.S. (1987). Seed germination of wild mustard (*Sinapis arvensis*): factors required to break primary dormancy. *Canadian Journal of Botany* 65:849-852.

GOUVÊA, C.M.C.P., SOUZA, J.F., MAGALHÃES, A.C.N., MARTINS, I.S. (1997). NO·-releasing substances that induce growth elongation in maize root segments. *Plant Growth Regulation* 21:183-187.

GOW, A.J., ISCHIROPOULOS, H. (2001). Nitric oxide chemistry and cellular signaling. *Journal of Cellular Physiology* 187:277-282.

GRANT, C.D., KOCH, J.M. (1997). Ecological aspects of soil seed-banks in relation to bauxite mining. II. Twelve year old rehabilitated mines. *Australian Journal of Ecology* 22:177-184.

GROOT, S.P.C., KARSSEN, C.M. (1987). Gibberellins regulate seed germination in tomato by endosperm weakening: a study with gibberellin deficient mutants. *Planta* 171:525-531.

GUILLÉN, M.D., MANZANOS, M.J., ZABALA, L. (1995). Study of a commercial liquid smoke flavoring by means of gas chromatography/mass spectrometry and Fourier transform infrared spectroscopy. *Journal of Agricultural and Food Chemistry* 43:463-468.

GUILLÉN, M.D., IBARGOITIA, M.L. (1996). Relationships between the maximum temperature reached in the smoke generation processes from *Vitis vinifera* L. shoot sawdust and composition of the aqueous smoke flavoring preparations obtained. *Journal of Agricultural and Food Chemistry* 44:1302-1307.

GUILLÉN, M.D., IBARGOITIA, M.L. (1999). Influence of the moisture content on the composition of the liquid smoke produced in the pyrolysis process of *Fagus sylvatica* L. wood. *Journal of Agricultural and Food Chemistry* 47:4126-4136.

HABER, A.H. (1965). Heat inactivation of gibberellic acid-sensitivity of lettuce seed germination. *Plant and Cell Physiology* 6:565-569.

HANES, T.L., JONES, H.W. (1967). Postfire chaparral succession in southern California. *Ecology* 48:257-264.

HENDRICKS, S.B., BORTHWICK, H.A. (1967). The function of phytochrome in regulation of plant growth. *Proceedings of the National Academy of Sciences of the USA* 58:2125-2130.

HENDRICKS, S.B., TAYLORSON, R.B. (1976). Variation in germination and amino acid leakage of seeds with temperature related to membrane phase change. *Plant Physiology* 58:7-11.

HENIG-SEVER, N., ESHEL, A., NE'EMAN, G. (1996). pH and osmotic potential of pine ash as post-fire germination inhibitors. *Physiologia Plantarum* 96:71-76.

HESS, C.E. (1964). A physiological analysis of root initiation in easy and difficult-to-root cuttings. *Proceedings of the 16th International Horticultural Congress* 4:375-381.

HICKEY, B., VAN JAARSVELD, E. (1995). Propagation of three rare and endangered vygies. *British Cactus and Succulent Journal* 13:141-146.

HILLHORST, H.W.M., KARSSEN, C.M. (1992). Seed dormancy and germination: the role of abscisic acid and gibberellins and the importance of hormone mutants. *Plant Growth Regulation* 11:225-238.

HILTON, J.R. (1984). The influence of light and potassium nitrate on the dormancy and germination of *Avena fatua* L. (wild oat) seed and its ecological significance. *New Phytologist* 96:31-34.

HOLLEY, R.A., PATEL, D. (2005). Improvement in shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food Microbiology* 22:273-292.

HOLMES, J. (1986). Orchids and fire. *South African Orchid Journal* 17:8-9.

HOLMES, P.M., RICHARDSON, D.M. (1999). Protocols for restoration based on knowledge of recruitment dynamics, community structure and ecosystem function: perspectives from South African fynbos. *Restoration Ecology* 7:215-231.

HOLMES, P.M., RICHARDSON, D.M., VAN WILGEN, B.W., GELDERBLOM, C. (2000). Recovery of South African fynbos vegetation following alien woody plant clearing and fire: implications for restoration. *Austral Ecology* 25:631-639.

HOLMES, P.M., NEWTON, R.J. (2004). Patterns of seed persistence in South African fynbos. *Plant Ecology* 172:143-158.

HORTON, J.S., KRAEBEL, C.J. (1955). Development of vegetation after fire in the chamise chaparral of southern California. *Ecology* 36:244-262.

HSIAO, A.I., VIDAVER, W. (1971). Seed water content in relation to phytochrome-mediated germination of lettuce seeds (*Lactuca sativa* L. var. Grand Rapids). *Canadian Journal of Botany* 49:111-115.

HSIAO, A.I., QUICK, W.A. (1997). Roles of soluble sugars in protecting phytochrome- and gibberellin A_3-mediated germination control in skotodormant seeds. *Journal of Plant Growth Regulation* 16:141-146.

HUMPHREY, A.J., BEALE, M.H. (2006). Strigol: biogenesis and physiological activity. *Phytochemistry* 67:636-640.

HUNG, K.T., CHANG, C.J., KAO, C.H. (2002). Paraquat toxicity is reduced by nitric oxide in rice leaves. *Journal of Plant Physiology* 159:159-166.

IKUMA, H., THIMANN, K.V. (1959). Photosensitive site in lettuce seeds. *Science* 130:568-569.

IKUMA, H., THIMANN, K.V. (1960). Action of gibberellic acid on lettuce seed germination. *Plant Physiology* 35:557-566.

IKUMA, H., THIMANN, K.V. (1963). The role of the seed-coats in germination of photosensitive lettuce seeds. *Plant and Cell Physiology* 4:169-185.

IKUMA, H. (1964). The effects of temperature on the germination and radicle growth of photosensitive lettuce seed. *Plant and Cell Physiology* 5:429-439.

IMANISHI, H., FORTANIER, E.J. (1982/3). Effects of exposing Freesia corms to ethylene or smoke on dormancy-breaking and flowering. *Scientia Horticulturae* 18:381-389.

IMANISHI, H. (1983). Effects of exposure of bulbs to smoke and ethylene on flowering of *Narcissus tazetta* cultivar 'Grand Soleil d'Or'. *Scientia Horticulturae* 21:173-180.

INOUE, Y., NAGASHIMA, H. (1991). Photoperceptive site in phytochrome-mediated lettuce (*Lactuca sativa* L. cv. Grand Rapids) seed germination. *Journal of Plant Physiology* 137:669-673.

JÄGER, A.K., LIGHT, M.E., VAN STADEN, J. (1996). Effects of source of plant material and temperature on the production of smoke extracts that promote germination of light-sensitive lettuce seeds. *Environmental and Experimental Botany* 36:421-429.

JÄGER, A.K., RABE, T., VAN STADEN, J. (1996). Food-flavouring smoke extracts promote seed germination. *South African Journal of Botany* 62:282-284.

JÄGER, A.K., STRYDOM, A., VAN STADEN, J. (1996). The effect of ethylene, octanoic acid and a plant-derived smoke extract on the germination of light-sensitive lettuce seeds. *Plant Growth Regulation* 19:197-201.

JÄGER, A.K., VAN STADEN, J. (2002). Soluble sugars in light-sensitive Grand Rapids lettuce seeds treated with red light, gibberellic acid and a plant-derived smoke extract. *South African Journal of Botany* 68:404-407.

JAKAB, E., LIU, K., MEUZELAAR, H.L.C. (1997). Thermal decomposition of wood and cellulose in the presence of solvent vapors. *Industrial and Engineering Chemistry Research* 36:2087-2095.

JAMIESON, H., BROWN, N. (1995). Restios with garden potential. *Pacific Horticulture* 56:36-41.

JONES, C.S., SCHLESINGER, W.H. (1980). *Emmenanthe penduliflora*: further consideration of the germination response. *Madroño* 27:122-125.

JUSAITIS, M., POLOMKA, L., SORENSEN, B. (2004). Habitat specificity, seed germination and experimental translocation of the endangered herb *Brachycome muelleri* (Asteraceae). *Biological Conservation* 116:251-266.

KAHN, A., GOSS, J.A., SMITH, D.E. (1957). Effect of gibberellin on germination of lettuce seed. *Science* 125:645-646.

KAHN, A. (1960). An analysis of "dark-osmotic inhibition" of germination of lettuce seeds. *Plant Physiology* 35:1-7.

KAMINSKY, R. (1981). The microbial origin of the allelopathic potential of *Adenostoma fasciculatum* H & A. *Ecological Monographs* 51:365-382.

KARSSEN, C.M., ZAGÓRSKI, S., KĘPCZYŃSKI, J., GROOT, S.P.C. (1989). Key role for endogenous gibberellins in the control of seed germination. *Annals of Botany* 63:71-80.

KEELEY, J.E. (1984). Factors affecting germination of chaparral seeds. *Bulletin of the Southern California Academy of Sciences* 83:113-120.

KEELEY, J.E., NITZBERG, M.E. (1984). Role of charred wood in the germination of the chaparral herbs *Emmenanthe penduliflora* (Hydrophyllaceae) and *Eriophyllum confertiflorum* (Asteraceae). *Madroño* 31:208-218.

KEELEY, J.E., MORTON, B.A., PEDROSA, A., TROTTER, P. (1985). Role of allelopathy, heat and charred wood in the germination of chaparral herbs and suffrutescents. *Journal of Ecology* 73:445-458.

KEELEY, J.E. (1986). Seed germination patterns of *Salvia mellifera* in fire-prone environments. *Oecologia* 71:1-5.

KEELEY, S.C., PIZZORNO, M. (1986). Charred wood stimulated germination of two fire-following herbs of the California chaparral and the role of hemicellulose. *American Journal of Botany* 73:1289-1297.

KEELEY, J.E. (1987). Role of fire in seed germination of woody taxa in California chaparral. *Ecology* 68:434-443.

KEELEY, J.E., KEELEY, S.C. (1987). Role of fire in the germination of chaparral herbs and suffrutescents. *Madroño* 34:240-249.

KEELEY, J.E. (1993). Smoke-induced flowering in the fire-lily *Cyrtanthus ventricosus*. *South African Journal of Botany* 59:638-639.

KEELEY, J.E., FOTHERINGHAM, C.J. (1997). Trace gas emissions and smoke-induced seed germination. *Science* 276:1248-1250.

KEELEY, J.E., FOTHERINGHAM, C.J. (1998a). Mechanism of smoke-induced seed germination in a post-fire chaparral annual. *Journal of Ecology* 86:27-36.

KEELEY, J.E., FOTHERINGHAM, C.J. (1998b). Smoke-induced seed germination in California chaparral. *Ecology* 79:2320-2336.

KEELEY, J.E., BAER-KEELEY, M. (1999). Role of charred wood, heat-shock, and light in germination of postfire phrygana species from the eastern Mediterranean basin. *Israel Journal of Plant Sciences* 47:11-16.

KEELEY, J.E., FOTHERINGHAM, C.J. (2000). Role of fire in regeneration from seed. In: *Seeds: The Ecology of Regeneration in Plant Communities*. Second Edition. Ed: Fenner, M. CABI Publishing, Wallingford, U.K. ISBN 0-85199-432-6. pp 311-330.

KEELEY, J.E., MCGINNIS, T.W., BOLLENS, K.A. (2005). Seed germination of Sierra Nevada postfire chaparral species. *Madroño* 52:175-181.

KEITH, D.A. (1997). Combined effects of heat shock, smoke and darkness on germination of *Epacris stuartii* Stapf., an endangered fire-prone Australian shrub. *Oecologia* 112:340-344.

KENDRICK, R.E., RUSSELL, J.H. (1975). Photomanipulation of phytochrome in lettuce seeds. *Plant Physiology* 56:332-334.

KENNY, B.J. (2000). Influence of multiple fire-related germination cues on three Sydney *Grevillea* (Proteaceae) species. *Austral Ecology* 25:664-669.

KĘPCZYŃSKI, J., BIAŁECKA, B., LIGHT, M.E., VAN STADEN, J. (2006). Regulation of *Avena fatua* seed germination by smoke solutions, gibberellin A_3 and ethylene. *Plant Growth Regulation* 49:9-16.

KHAN, A.A. (1968). Inhibition of gibberellic acid-induced germination by abscisic acid and reversal by cytokinins. *Plant Physiology* 43:1463-1465.

KOORNNEEF, M., BENTSINK, L., HILHORST, H. (2002). Seed dormancy and germination. *Current Opinion in Plant Biology* 5:33-36.

KOPYRA, M., GWÓŹDŹ, E.A. (2003). Nitric oxide stimulates seed germination and counteracts the inhibitory effect of heavy metals and salinity on root growth of *Lupinus luteus*. *Plant Physiology and Biochemistry* 41:1011-1017.

KROCK, B., SCHMIDT, S., HERTWECK, C., BALDWIN, I.T. (2002). Vegetation-derived abscisic acid and four terpenes enforce dormancy in seeds of the post-fire annual, *Nicotiana attenuata*. *Seed Science Research* 12:239-252.

KRUGER, F.J., BIGALKE, R.C. (1984). Fire in fynbos. In: *Ecological Effects of Fire in South African Ecosystems. Ecological Studies 48.* Eds: Booysen, P. de V., Tainton, N.M. Springer-Verlag, Berlin, Germany. ISBN 3-540-13501-4. pp 67-114.

KUCERA, B., COHN, M.A., LEUBNER-METZGER, G. (2005). Plant hormone interactions during seed dormancy release and germination. *Seed Science Research* 15:281-307.

KULKARNI, M.G., SPARG, S.G., VAN STADEN, J. (2006). Dark conditioning, cold stratification and a smoke-derived compound enhance the germination of *Eucomis autumnalis* subsp. *autumnalis* seeds. *South African Journal of Botany* 72:157-162.

LAMATTINA, L., GARCÍA-MATA, C., GRAZIANO, M., PAGNUSSAT, G. (2003). Nitric oxide: The versatility of an extensive signal molecule. *Annual Review of Plant Biology* 54:109-136.

LAMONT, B.B., LE MAITRE, D.C., COWLING, R.M., ENRIGHT, N.J. (1991). Canopy seed storage in woody plants. *Botanical Review* 57:277-317.

LAMONT, B.B., GROOM, P.K. (1998). Seed and seedling biology of the woody-fruited Proteaceae. *Australian Journal of Botany* 46:387-406.

LE HOUÉROU, H.N. (1981). Impact of man and his animals on Mediterranean vegetation. In: *Ecosystems of the World: 11. Mediterranean-type Shrublands.* Eds: di Castri, F., Goodall, D.W., Specht, R.L. Elsevier Scientific Publishing Company, Amsterdam. ISBN 0-444-41858-X. pp 479-521.

LE MAITRE, D.C., BROWN, P.J. (1992). Life cycles and fire-stimulated flowering in geophytes. In: *Fire in South African Mountain Fynbos: Ecosystem, Community and Species Response at Swartboskloof.* Eds: van Wilgen, B.W., Richardson, D.M., Kruger, F.J., van Hensbergen, H.J. Springer-Verlag, Berlin Germany. ISBN 0-387-53301-X. pp 145-160.

LEUNG, D.W.M., BEWLEY, J.D. (1981). Red-light- and gibberellic-acid-enhanced α-galactosidase activity in germinating lettuce seeds, cv. Grand Rapids. *Planta* 152:436-441.

LEVY, S. (2005). Rekindling native fires. *BioScience* 55:303-308.

LEVYNS, M.R. (1935). Germination in some South African seeds. *Journal of South African Botany* 1:161-170.

LEWAK, S., KHAN, A.A. (1977). Mode of action of gibberellic acid and light on lettuce seed germination. *Plant Physiology* 60:575-577.

LEYSER, O., DAY, S. (2003). *Mechanisms in Plant Development.* Blackwell Science Ltd, Oxford, UK. ISBN 0-86542-742-9. pp 1-241.

LIGHT, M.E., GARDNER, M.J., JÄGER, A.K., VAN STADEN, J. (2002). Dual regulation of seed germination by smoke solutions. *Plant Growth Regulation* 37:135-141.

LIGHT, M.E., VAN STADEN, J. (2003). The nitric oxide specific scavenger carboxy-PTIO does not inhibit smoke stimulated germination of Grand Rapids lettuce seeds. *South African Journal of Botany* 69:217-219.

LIGHT, M.E., VAN STADEN, J. (2004). The potential of smoke in seed technology. *South African Journal of Botany* 70:97-101.

LIGHT, M.E., BURGER, B.V., VAN STADEN, J. (2005). Formation of a seed germination promoter from carbohydrates and amino acids. *Journal of Agricultural and Food Chemistry* 53:2936-5942.

LLOYD, M.V., DIXON, K.W., SIVASITHAMPARAM, K. (2000). Comparative effects of different smoke treatments on germination of Australian native plants. *Austral Ecology* 25:610-615.

LLOYD, M.V., BARNETT, G., DOHERTY, M.D., JEFFREE, R.A., JOHN, J., MAJER, J.D., OSBORNE, J.M., NICHOLS, O.G. (2002). Managing the impacts of the Australian minerals industry on biodiversity. Final report by the Australian Centre for Mining Environmental Research. Internet address: http://www.iied.org/mmsd/mmsd_pdfs/098_acmer.pdf, Accessed 11/08/03.

LUSTRE, A.O., ISSENBERG, P. (1969). Volatile components of hardwood sawdust smoke: components of phenolic fraction. *Journal of Agricultural and Food Chemistry* 17:1387-1393.

LYNDS, G.Y., BALDWIN, I.T. (1998). Fire, nitrogen, and defensive plasticity in *Nicotiana attenuata*. *Oecologia* 115:531-540.

MAGA, J.A. (1988). *Smoke in Food Processing*. CRC Press, Boca Raton, USA. ISBN 0-8493-5155-3.

MANGNUS, E.M., ZWANENBURG, B. (1992). Tentative molecular mechanism for germination stimulation of *Striga* and *Orobanche* seeds by strigol and its synthetic analogues. *Journal of Agricultural and Food Chemistry* 40:1066-1070.

MARGARIS, N.S. (1981). Adaptive strategies in plants dominating Mediterranean-type ecosystems. In: *Ecosystems of the World: 11. Mediterranean-type Shrublands*. Eds: di Castri, F., Goodall, D.W., Specht, R.L. Elsevier Scientific Publishing Company, Amsterdam. ISBN 0-444-41858-X. pp 309-315.

MARTIN, A.R.H. (1966). The plant ecology of the Grahamstown Nature Reserve: II. Some effects of burning. *Journal of South African Botany* 32:1-39.

MATUSOVA, R., RANI, K., VERSTAPPEN, F.W.A., FRANSSEN, M.C.F., BEALE, M.H., BOUWMEESTER, H.J. 2005. The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanche* spp. are derived from the carotenoid pathway. *Plant Physiology* 139:920-934.

MAURON, J. (1981). The Maillard reaction in food: A critical review from the nutritional standpoint. In: *Maillard Reactions in Food: Chemical, Physiological and Technological Aspects*. Ed: Eriksson, C. Pergamon Press, Oxford. ISBN 0-08-025496-9. pp 5-35.

McARTHUR, J.A. (1978). Light effects upon dry lettuce seeds. *Planta* 144:1-5.

McCARTAN, S.A., RAMAROSANDRATANA, A.V., VAN STADEN, J. (2004). *In vitro* germination and propagation of the ornamental restio, *Ischyrolepis subverticillata*. *South African Journal of Botany* 70:326-328.

McPHERSON, J.K., MULLER, C.H. (1969). Allelopathic effects of *Adenostoma fasciculatum*, "chamise", in the California chaparral. *Ecological Monographs* 39:177-198.

MERRITT, D.J., KRISTIANSEN, M., FLEMATTI, G.R., TURNER, S.R., GHISALBERTI, E.L., TRENGOVE, R.D., DIXON, K.W. (2006). Effects of a butenolide present in smoke on light-mediated germination of Australian *Asteraceae*. *Seed Science Research* 16:29-35.

MINORSKY, P.V. (2002). The hot and the classic. Smoke-induced germination. *Plant Physiology* 128:1167-1168.

MODI, A.T. (2002). Indigenous storage method enhances seed vigour of traditional maize. *South African Journal of Science* 98:138-139.

MODI, A.T. (2004). Short-term preservation of maize landrace seed and taro propagules using indigenous storage methods. *South African Journal of Botany* 70:16-23.

MOLL, E.J., GUBB, A.A. (1981). Aspects of the ecology of *Staavia dodii* in the South Western Cape of South Africa. In: *The Biological Aspects of Rare Plant Conservation*. Ed: Synge, H. John Wiley & Sons, Chichester. ISBN 0-471-28004-6. pp 331-342.

MORENO, J.M., OECHEL, W.C. (1991). Fire intensity effects on germination of shrubs and herbs in southern California chaparral. *Ecology* 72:1993-2004.

MORRIS, E.C. (2000). Germination response of seven east Australian *Grevillea* species (Proteaceae) to smoke, heat exposure and scarification. *Australian Journal of Botany* 48:179-189.

MORRIS, E.C., TIEU, A., DIXON, K. (2000). Seed coat dormancy in two species of *Grevillea* (Proteaceae). *Annals of Botany* 86:771-775.

MUÑOZ, M.R., FUENTES, E.R. (1989). Does fire induce shrub germination in the Chilean matorral? *Oikos* 56:177-181.

MUSIL., C.F. (1991). Seed bank dynamics in sand plain lowland fynbos. *South African Journal of Botany* 57:131-142.

MUSIL, C.F., DE WITT, D.M. (1991). Heat-stimulated germination in two Restionaceae species. *South African Journal of Botany* 57:175-176.

NAKAMINAMI, K., SAWADA, Y., SUZUKI, M., KENMOKU, H., KAWAIDE, H., MITSUHASHI, W., SASSA, T., INOUE, Y., KAMIYA, Y., TOYOMASU, T. (2003). Deactivation of gibberellin by 2-oxidation during germination of photoblastic lettuce seeds. *Bioscience, Biotechnology and Biochemistry* 67:1551-1558.

NAVEH, Z. (1975). The evolutionary significance of fire in the Mediterranean region. *Vegetatio* 29:199-208.

NEILL, S.J., DESIKAN, R., HANCOCK, J.T. (2003). Nitric oxide signalling in plants. *New Phytologist* 159:11-35.

NEWTON, R.J., BOND, W.J., FARRANT, J.M. (2006). Effects of seed storage and fire on germination in the nut-fruited Restionaceae species, *Cannomois virgata*. *South African Journal of Botany* 72:177-180.

NORITAKE, T., KAWAKITA, K., DOKE, N. (1996). Nitric oxide induces phytoalexin accumulation in potato tuber tissues. *Plant Cell Physiology* 37:113-116.

NURSTEN, H.E., O'REILLY, R. (1986). The complexity of the Maillard reaction as shown by a xylose-glycine model system. In: *Amino-carbonyl Reactions in Food and Biological Systems*. Eds: Fujimaki, M., Namiki, M., Hiromichi, K. Elsevier Science Publishers, Amsterdam. ISBN 0-444-99510-2. pp 17-28.

OBROUCHEVA, N.V., ANTIPOVA, O.V. (1997). Physiology of the initiation of seed germination. *Russian Journal of Plant Physiology* 44:250-264.

ODION, D.C., DAVIS, F.W. (2000). Fire, soil heating, and the formation of vegetation patterns in chaparral. *Ecological Monographs* 70:149-169.

OLIVIER, W., WERNER, W. (1980). The genus *Cyrtanthus* Ait. *Veld and Flora* 66:78-81.

PAASONEN, M., HANNUKKALA, A., RÄMÖ, S., HAAPALA, H., HIETANIEMI, V. (2003). Smoke - a novel application of a traditional means to improve grain quality. *Nordic Association of Agricultural Scientists 22nd Congress, July 1-4 2003, Turku, Finland.* (Abstract)

PAGNUSSAT, G.C., SIMONTACCHI, M., PUNTARULO, S., LAMATTINA, L. (2002). Nitric oxide is required for root organogenesis. *Plant Physiology* 129:954-956.

PARMETER, J.R., UHRENHOLDT, B. (1975). Some effects of pine-needle or grass smoke on fungi. *Phytopathology* 65:28-31.

PARSONS, R.F. (1981). *Eucalyptus* scrubs and shrublands. In: *Australian Vegetation*. Ed: Groves, R.H. Cambridge University Press, Cambridge. ISBN 0-521-23436-0. pp 227-252.

PÉREZ-FERNÁNDEZ, M.A., RODRÍGUEZ-ECHEVERRÍA, S. (2003). Effect of smoke, charred wood, and nitrogenous compounds on seed germination of ten species from woodland in central-western Spain. *Journal of Chemical Ecology* 29:237-251.

PIENAAR, U.D.V. (1995). Instant smoke seed primer stimulates the germination of vygie seeds. *Veld and Flora*. 81:93.

PIERCE, S.M., COWLING, R.M. (1991). Dynamics of soil-stored seed banks of six shrubs in fire-prone dune fynbos. *Journal of Ecology* 79:731-747.

PIERCE, S.M., MOLL, E.J. (1994). Germination ecology of six shrubs in fire-prone Cape fynbos. *Vegetatio* 110:25-41.

PIERCE, S.M., ESLER, K., COWLING, R.M. (1995). Smoke-induced germination of succulents (Mesembryanthemaceae) from fire-prone and fire-free habitats in South Africa. *Oecologia* 102:520-522.

PLUMMER, J.A., ROGERS, A.D., TURNER, D.W., BELL, D.T. (2001). Light, nitrogenous compounds, smoke and GA_3 break dormancy and enhance germination in the Australian everlasting daisy, *Shoenia filifolia* subsp. *subulifolia*. *Seed Science and Technology* 29:321-330.

PONS, T.L. (2000). Seed responses to light. In: *Seeds: The Ecology of Regeneration in Plant Communities*. Second Edition. Ed: Fenner, M. CABI Publishing, Wallingford, U.K. ISBN 0-85199-432-6. pp 237-260.

POPAY, A.I., ROBERTS, E.H. (1970). Factors involved in the dormancy and germination of *Capsella bursa-pastoris* (L.) Medik., *Senecio vulgaris* L. *Journal of Ecology* 58:103-122.

POWELL, A.D., LEUNG, D.W.M., BEWLEY, J.D. (1983). Long-term storage of dormant Grand Rapids lettuce seeds in the imbibed state: physiological and metabolic changes. *Planta* 159:182-188.

PRESTON, C.A., BALDWIN, I.T. (1999). Positive and negative signals regulate germination in the post-fire annual, *Nicotiana attenuata*. *Ecology* 80:481-494. Erratum, 81:293.

PRESTON, C.A., BECKER, R., BALDWIN, I.T. (2004). Is 'NO' news good news? Nitrogen oxides are not components of smoke that elicits germination in two smoke-stimulated species, *Nicotiana attenuata* and *Emmenanthe penduliflora*. *Seed Science Research* 14:73-79.

RAO, V.S., SANKHLA, N., KHAN, A.A. (1975). Additive and synergistic effects of kinetin and ethrel on germination, thermodormancy, and polyribosome formation in lettuce seeds. *Plant Physiology* 56:263-266.

RAO, V.S., BRAUN, J.W., KHAN, A.A. (1976). Promotive effects of organic solvents and kinetin on dark germination of lettuce seeds. *Plant Physiology* 57:446-449.

RAZANAMANDRANTO, S., TIGABU, M., SAWADOGO, L., ODÉN, P.C. (2005). Seed germination of eight savanna-woodland species from West Africa in response to different cold smoke treatments. *Seed Science and Technology* 33:315-328.

READ, T.R., BELLAIRS, S.M. (1999). Smoke affects the germination of native grasses of New South Wales. *Australian Journal of Botany* 47:563-576.

READ, T.R., BELLAIRS, S.M., MULLIGAN, D.R., LAMB, D. (2000). Smoke and heat effects on soil seed bank germination for the re-establishment of a native forest community in New South Wales. *Austral Ecology* 25:48-57.

REYES, O., CASAL, M. (1998). Germination of *Pinus pinaster, P. radiata* and *Eucalyptus globulus* in relation to the amount of ash produced in forest fires. *Annales des Sciences Forestieres* 55:837-845.

RICHARDSON, D.M., MACDONALD, I.A.W., HOFFMANN, J.H., HENDERSON, L. (1997). Alien plant invasion. In: *Vegetation of Southern Africa*. Eds: Cowling, R.M., Richardson, D.M., Pierce, S.M. Cambridge University Press, Cambridge. ISBN 0-521-57142-1. pp 535-570.

ROBERTS, E.H., BENJAMIN, S.K. (1979). The interaction of light, nitrate and alternating temperature on the germination of *Chenopodium album*, *Capsella bursa-pastoris* and *Poa annua* before and after chilling. *Seed Science and Technology* 7:379-392.

ROCHE, S., DIXON, K.W., PATE, J.S. (1997). Seed ageing and smoke: Partner cues in the amelioration of seed dormancy in selected Australian native species. *Australian Journal of Botany* 45:783-815.

ROCHE, S., KOCH, J., DIXON, K.W. (1997). Smoke-enhanced seed germination for mine rehabilitation in the south-west of Western Australia. *Restoration Ecology* 5:191-203.

ROCHE, S., DIXON, K.W., PATE, J.S. (1998). For everything a season: Smoke-induced seed germination and seedling recruitment in Western Australian *Banksia* woodland. *Australian Journal of Ecology* 23:111-120.

ROKICH, D.P., DIXON, K.W., SIVASITHAMPARAM, K., MENEY, K.A. (2002). Smoke, mulch, and seed broadcasting effects on woodland restoration in Western Australia. *Restoration Ecology* 10:185-194.

ROTH-BEJERANO, N., SEDEE, N.J.A., VAN DER MEULEN, R.M., WANG, M. (1999). The role of abscisic acid in germination of light-sensitive and light-insensitive lettuce seeds. *Seed Science Research* 9:129-134.

ROY, J., ARIANOUTSOU-FARAGGITAKI, M. (1985). Light quality as the environmental trigger for germination of the fire-promoted species *Sarcopoterium spinosum* L. *Flora* 177:345-349.

SAINI, H.S., BASSI, P.K., SPENCER, M.S. (1985). Interactions among ethephon, nitrate, and after-ripening in the release of dormancy of wild oat (*Avena fatua*) seed. *Weed Science* 43:43-47.

SAKUMA, H., MUNAKATA, S., SUGAWARA, S. (1981). Volatile products of cellulose pyrolysis. *Agricultural and Biological Chemistry* 45:443-451.

SCHEIBE, J., LANG, A. (1965). Lettuce seed germination: Evidence for a reversible light-induced increase in growth potential and for phytochrome mediation of the low temperature effect. *Plant Physiology* 40:485-492.

SCHEIBE, J., LANG, A. (1967). Lettuce seed germination: A phytochrome-mediated increase in the growth rate of lettuce seed radicles. *Planta* 72:348-354.

SCHELPE, E.A. (1970). Fire induced flowering among indigenous South African orchids. *South African Orchid Journal* June:21.

SCHWACHTJE, J., BALDWIN, I.T. (2004). Smoke exposure alters endogenous gibberellin and abscisic acid pools and gibberellin sensitivity while eliciting germination in the post-fire annual, *Nicotiana attenuata*. *Seed Science Research* 14:51-60.

SEGURA, A.M., HOLMGREN, M., ANABALÓN, J.J., FUENTES, E.R. (1998). The significance of fire intensity in creating local patchiness in the Chilean matorral. *Plant Ecology* 139:259-264.

SENARATNA, T., DIXON, K.W., BUNN, E., TOUCHELL, D. (1999). Smoke-saturated water promotes somatic embryogenesis in geranium. *Plant Growth Regulation* 28:95-99.

SINK, J.D. (1979). Effects of smoke processing on muscle food product characteristics. *Food Technology* 33:72-83.

SMALL, J.G.C., GUTTERMAN, Y. (1991). Evidence for inhibitor involvement in thermodormancy of Grand Rapids lettuce seeds. *Seed Science Research* 1:263-267.

SMITH, M.A., BELL, D.T., LONERAGAN, W.A. (1999). Comparative seed germination ecology of *Austrostipa compressa* and *Ehrharta calycina* (Poaceae) in a western Australian *Banksia* woodland. *Australian Journal of Ecology* 24:35-42.

SOFOS, J.N., MAGA, J.A., BOYLE, D.L. (1988). Effect of ether extracts from condensed wood smokes on the growth of *Aeromonas hydrophila* and *Staphylococcus aureus*. *Journal of Food Science* 53:1840-1843.

SOKAL, R.R., ROHLF, F.J. (1987). *Introduction to Biostatistics*, 2nd Edition. W.H. Freeman and Company, New York. ISBN 0-716-71805-7.

SPARG, S.G., KULKARNI, M.G., LIGHT, M.E., VAN STADEN, J. (2005). Improving seedling vigour of indigenous medicinal plants with smoke. *Bioresource Technology* 96:1323-1330.

SPARG, S.G., KULKARNI, M.G., VAN STADEN, J. (2006). Aerosol smoke and smoke-water stimulation of seedling vigor of a commercial maize cultivar. *Crop Science* 46:1336-1340.

SPECHT, R.L., RAYSON, P., JACKMAN, M.E. (1958). Dark island heath (ninety-mile plain, south Australia). VI. Pyric succession: changes in composition, coverage, dry weight, and mineral nutrient status. *Australian Journal of Botany* 6:59-88, and 4 plates.

STEWART, R.R.C., BERRIE, A.M.M. (1979). Effect of temperature on the short chain fatty acid-induced inhibition of lettuce seed germination. *Plant Physiology* 63:61-62.

STOCK, W.D., LEWIS, O.A.M. (1986). Soil nitrogen and the role of fire as a mineralizing agent in a South African coastal fynbos ecosystem. *Journal of Ecology* 74:317-328.

STRYDOM, A., JÄGER, A.K., VAN STADEN, J. (1996). The effect of plant-derived smoke extract, N6-benzyladenine and gibberellic acid on the thermodormancy of lettuce seeds. *Plant Growth Regulation* 19:97-100.

SUÑEN, E., FERNANDEZ-GALIAN, B., ARISTIMUÑO, C. (2001). Antibacterial activity of smoke wood condensates against *Aeromonas hydrophila*, *Yersinia enterocolitica* and *Listeria monocytogenes* at low temperature. *Food Microbiology* 18:387-393.

SUTCLIFFE, M.A., WHITEHEAD, C.S. (1995). Role of ethylene and short-chain saturated fatty acids in the smoke-stimulated germination of *Cyclopia* seed. *Journal of Plant Physiology* 145:271-276.

TANG, Y., BOULTER, S.L., KITCHING, R.L. (2003). Heat and smoke effects on the germination of seeds from soil seed banks across forest edges between subtropical rainforest and eucalypt forest at Lamington National Park, south-eastern Queensland, Australia. *Australian Journal of Botany* 51:227-237.

TAO, K.-L., KHAN, A.A. (1979). Changes in the strength of lettuce endosperm during germination. *Plant Physiology* 63:126-128.

TAYLOR, H.C. (1978). Capensis. In: *Biogeography and Ecology of Southern Africa. Volume 1.* Ed: Werger, M.J.A. Junk, The Hague. pp 171-229. ISBN 90-6193-083-9.

TAYLOR, J.L.S., VAN STADEN, J. (1996). Root initiation in *Vigna radiata* (L.) Wilczek hypocotyl cuttings is stimulated by smoke-derived extracts. *Plant Growth Regulation* 18:165-168.

TAYLOR, J.L.S., VAN STADEN, J. (1998). Plant-derived smoke solutions stimulate the growth of *Lycopersicon esculentum* roots *in vitro*. *Plant Growth Regulation* 26:77-83.

THANOS, C.A., RUNDEL, P.W. (1995). Fire-followers in chaparral: nitrogenous compounds trigger seed germination. *Journal of Ecology* 83:207-216.

THOMAS, T.H. (1992). Some reflections on the relationship between endogenous hormones and light-mediated seed dormancy. *Plant Growth Regulation* 11:239-248.

THOMAS, T.H., VAN STADEN, J. (1995). Dormancy break of celery (*Apium graveolens* L.) seeds by plant-derived smoke extract. *Plant Growth Regulation* 17:195-198.

THOMAS, T.H., DAVIES, I. (2002). Responses of dormant heather (*Calluna vulgaris*) seeds to light, temperature, chemical and advancement treatments. *Plant Growth Regulation* 37:23-29.

THOMAS, P.B., MORRIS, E.C., AULD, T.D. (2003). Interactive effects of heat shock and smoke on germination of nine species forming soil seed banks within the Sydney region. *Austral Ecology* 28:674-683.

THOMPSON, K., GRIME, J.P. (1979). Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. *Journal of Ecology* 67:893-921.

THORNTON, M.A., THOMAS, T.H., PETERS, N.C.B. (1999). The promotive effect of combustion products from plant vegetation on the release of seeds from dormancy. *Plant Growth Regulation* 28:129-132.

TIERNEY, D.A. (2006). The effect of fire-related germination cues on the germination of a declining forest understorey species. *Australian Journal of Botany* 54:297-303.

TIEU, A., DIXON, K.A., SIVASITHAMPARAM, K., PLUMMER, J.A., SIELER, I.M. (1999). Germination of four species of native Western Australian plants using plant-derived smoke. *Australian Journal of Botany* 47:207-219.

TIEU, A., DIXON, K.W., MENEY, K.A., SIVASITHAMPARAM, K. (2001a). The interaction of heat and smoke in the release of seed dormancy in seven species from Southwestern Western Australia. *Annals of Botany* 88:259-265.

TIEU, A., DIXON, K.W., MENEY, K.A., SIVASITHAMPARAM, K. (2001b). Interaction of soil burial and smoke on germination patterns in seeds of selected Australian native plants. *Seed Science Research* 11:69-76.

TIEU, A., DIXON, K.W., MENEY, K.A., SIVASITHAMPARAM, K., BARRETT, R.L. (2001). Spatial and developmental variation in seed dormancy characteristics in the fire-responsive species *Anigozanthos manglesii* (Haemodoraceae) from western Australia. *Annals of Botany* 88:19-26.

TODOROVIĆ, S., GIBA, Z., ŽIVKOVIĆ, S., GRUBIŠIĆ, D., KONJEVIĆ, R. (2005). Stimulation of empress tree seed germination by liquid smoke. *Plant Growth Regulation* 47:141-148.

TOMPSETT, A.A. (1985). Dormancy breaking in bulbs by burning over. *The Plantsman* 7:40-52.

TOOLE, E.H., HENDRICKS, S.B., BORTHWICK, H.A., TOOLE, V.K. (1956). Physiology of seed germination. *Annual Review of Plant Physiology* 7:299-324.

TOYOMASU, T., TSUJI, H., YAMANE, H., NAKAYAMA, M., YAMAGUCHI, I., MUROFUSHI, N., TAKAHASHI, N., INOUE, Y. (1993). Light effects on endogenous levels of gibberellins in photoblastic lettuce seeds. *Journal of Plant Growth Regulation* 12:85-90.

TOYOMASU, T., YAMANE, H., MUROFUSHI, N., INOUE, Y. (1994). Effects of exogenously applied gibberellin and red light on the endogenous levels of abscisic acid in photoblastic lettuce seeds. *Plant Cell Physiology* 35:127-129.

TOYOMASU, T., KAWAIDE, H., MITSUHASHI, W., INOUE, Y., KAMIYA, Y. (1998). Phytochrome regulates gibberellin biosynthesis during germination of photoblastic lettuce seeds. *Plant Physiology* 118:1517-1523.

TRABAUD, L. (1981). Man and fire: impacts on Mediterranean vegetation. In: *Ecosystems of the World: 11. Mediterranean-type Shrublands.* Eds: di Castri, F., Goodall, D.W., Specht, R.L. Elsevier Scientific Publishing Company, Amsterdam. ISBN 0-444-41858-X. pp 523-537.

TRAUB, H.P., COOPER, W.C., REECE, P.C. (1940). Inducing flowering in the pineapple, *Ananas sativus*. *Proceedings of the American Society for Horticultural Science* 37:521-525.

TYLER, C.M. (1995). Factors contributing to postfire seedling establishment in chaparral: direct and indirect effects of fire. *Journal of Ecology* 83:1009-1020.

UYEMURA, S., IMANISHI, H. (1983). Effects of gaseous compounds in smoke on dormancy release in freesia corms. *Scientia Horticulturae* 20:91-99.

VAN DE VENTER, H.A., ESTERHUIZEN, A.D. (1988). The effects of factors associated with fire on seed germination of *Erica sessiliflora* and *E. hebecalyx* (Ericaceae). *South African Journal of Botany* 54:301-304.

VAN STADEN, J., DREWES, F.E., BROWN, N.A.C. (1995). Some chromatographic characteristics of germination stimulants in plant-derived smoke extracts. *Plant Growth Regulation* 17:241-249.

VAN STADEN, J., DREWES, F.E., JÄGER, A.K. (1995). The search for germination stimulants in plant-derived smoke extracts. *South African Journal of Botany* 61:260-263.

VAN STADEN, J., JÄGER, A.K., STRYDOM, A. (1995). Interaction between a plant-derived smoke extract, light and phytohormones on the germination of light-sensitive lettuce seeds. *Plant Growth Regulation* 17:213-218.

VAN STADEN, J., BROWN, N.A.C., JÄGER, A.K., JOHNSON, T.A. (2000). Smoke as a germination cue. *Plant Species Biology* 15:167-178.

VAN STADEN, J., JÄGER, A.K., LIGHT, M.E., BURGER, B.V. (2004). Isolation of the major germination cue from plant-derived smoke. *South African Journal of Botany* 70:654-659.

VAN STADEN, J., SPARG, S.G., KULKARNI, M.G., LIGHT, M.E. (2006). Post-germination effects of the smoke-derived compound 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one, and its potential as a preconditioning agent. *Field Crops Research* 98:98-105.

VAN WILGEN, B.W., LE MAITRE, D.C., COWLING, R.M. (1998). Ecosystem service, efficiency sustainability and equity: South Africa's Working for Water programme. *Trends in Ecology and Evolution* 13:378.

VERSCHAEVE, L., MAES, J., LIGHT, M.E., VAN STADEN, J. (2006). Genetic toxicity testing of 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one, an important biologically active compound from plant-derived smoke. *Mutation Research – Genetic Toxicology and Environmental Mutagenesis*. In press.

VIDAVER, W., HSIAO, A.I. (1974). Actions of gibberellic acid and phytochrome on the germination of Grand Rapids lettuce seeds. *Plant Physiology* 53:266-268.

VITT, S.M., HIMELBLOOM, B.H., CRAPO, C.A. (2001). Inhibition of *Listeria inocula* and *Listeria monocytogenes* in a laboratory medium and cold-smoked salmon containing liquid smoke. *Journal of Food Safety* 2:111-125.

WALCK, J.L., BASKIN, J.M., BASKIN, C.C. (1996). An ecologically and evolutionarily meaningful definition of a persistent seed bank in *Solidago*. *American Journal of Botany* 83 (supplement to 6):78-79.

WARD, S.C., KOCH, J.M., GRANT, C.D. (1997). Ecological aspects of soil seed-banks in relation to bauxite mining. I. Unmined jarrah forest. *Australian Journal of Ecology* 22:169-176.

WICKLOW, D.T. (1977). Germination response in *Emmenanthe penduliflora* (Hydrophyllaceae). *Ecology* 58:201-205.

WIGCHERT, S.C.M., ZWANENBURG, B. (1999). A critical account on the inception of *Striga* seed germination. *Journal of Agricultural and Food Chemistry* 47:1320-1325.

WILLIAMS, P.R., CONGDON, R.A., GRICE, A.C., CLARKE, P.J. (2003a). Effect of fire regime on plant abundance in a tropical eucalypt savanna of north-eastern Australia. *Austral Ecology* 28:327-338.

WILLIAMS, P.R., CONGDON, R.A., GRICE, A.C., CLARKE, P.J. (2003b). Fire-related cues break seed dormancy of six legumes of tropical eucalypt savannas in north-eastern Australia. *Austral Ecology* 28:507-514.

WILLS, T.J., READ, J. (2002). Effects of heat and smoke on germination of soil-stored seed in a south-eastern Australian sand heathland. *Australian Journal of Botany* 50:197-206.

YABIKU, Y.H., MARTINS, S.M., TAKAHASHI, M.Y. (1993). Levels of benzo(a)pyrene and other polycyclic aromatic hydrocarbons in liquid smoke flavour and some smoked foods. *Food Additives and Contaminants* 10:399-405.

YOSHIOKA, T., ENDO, T., SATOH, S. (1998). Restoration of seed germination at supraoptimal temperatures by fluridone, an inhibitor of abscisic acid biosynthesis. *Plant Cell Physiology* 39:307-312.

YOSHIOKA, T., GONAI, T., KAWAHARA, S., SATOH, S., HASHIBA, T. (2003). The regulation of the thermoinhibition of seed germination in winter annual plants by abscisic acid. In: *The Biology of Seeds: Recent Research Advances*. Eds: Nicolás, G., Bradford, K.J., Côme, D., Pritchard, H.W. CABI Publishing. ISBN 0-85199-653-1. pp 217-223.

ZAGÓRSKI, S., LEWAK, S. (1983). Interactions between hydrogen cyanide, gibberellin, abscisic acid and red light in germination of lettuce seeds. *Physiologia Plantarum* 59:95-98.

APPENDIX 1
List of species which show a germination response to smoke treatments

Species in which seed germination is stimulated by smoke, smoke solutions, charred wood or aqueous extracts of charred wood.

Habitat
Aus = Australia
CFR = Cape Floristic Region, South Africa
jf = jarrah forest
MB = Mediterranean Basin
NSW = New South Wales, Australia
phr = phrygana community
QL = Queensland, Australia
rm = rehabilitated mine soils
SA = South Africa
SCal = southern California
SN = Sierra Nevada
UK = United Kingdom
US = United States of America
WA = Western Australia

Treatment
acid = acid scarification
bcs = broadcast seed (rehabilitated mine soils)
CE = charate extract (extract from powdered charred wood)
CH = charate (powdered charred wood)
fs = foliar spray (smoke solution)
GA = gibberellins
PI = pre-imbibed/pre-treatment
S = smoke (treatment undefined)
SA = aerosol smoke
SC = smoke-derived germination compound, 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one
scr = scarification
SS = smoke-treated substrate (e.g. sand, filter paper)
ssb = soil seed bank
SW = smoke water
SWcls = commercial liquid smoke

Notes on germination data
Where possible, germination data are included. Certain studies represented results graphically, or a number of experiments were conducted on the same species. In these cases data are excluded. If no units are given, results represent percentage germination. For some species results are given as number of seedlings per gram of seed. For soil seed bank studies, figures represent the mean number of seedlings per square metre or per plot.

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Gymnospermae					
CUPRESSACEAE					
Widdringtonia cuppressiodes (syn. *W. nodiflora*)	CFR	S			BROWN and BOTHA, 1995
Actinostrobus acuminatus	WA	SA	~ 16	~ 84	DIXON, ROCHE and PATE, 1995 BELL, 1999
Callitris intratropica	Aus	SA	1.3 ± 1.3	13.9 ± 1.8	ROCHE, DIXON and PATE, 1997 BELL, 1999
Monocotyledonae					
ANTHERICACEAE					
Agrostocrinum scabrum	WA/rm	SA/storage SW/ssb SA/bcs	0 1.00 plot^{-1} 1.93 plot^{-1}	6.7 ± 0.9 3.17 plot^{-1} 5.7 plot^{-1}	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999
Arthropodium strictum	Aus	SA	29 ± 2.5	59.3 ± 3.2	ROCHE, DIXON and PATE, 1997 BELL, 1999
Caesia calliantha	Aus	SA	4 ± 0.9	10.9 ± 2	ROCHE, DIXON and PATE, 1997 BELL, 1999
Chamaescilla corymbosa	WA/rm	SA SA/ssb SW PI	~ 30 47.9 ± 2.7 48.5 0.10 m^{-2} 18.00 4m^{-2}	~ 40 85.6 ± 4.9 86.4 1.98 m^{-2} 32.39 4m^{-2} 76.11 4m^{-2} 60.78 4m^{-2}	DIXON, ROCHE and PATE, 1995 ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999 ADKINS, PETERS, PATERSON and NAVIE, 2003 ALLAN, ADKINS, PRESTON and BELLAIRS, 2004
Laxmannia omnifertilis	Aus	SA	22.1 ± 1.2	37.2 ± 1.4	ROCHE, DIXON and PATE, 1997 BELL, 1999
Laxmannia orientalis	Aus	SW/ssb			ENRIGHT and KINTRUP, 2001
Laxmannia sessiliflora	WA	SA	15 ± 2 plot^{-1}	82 ± 14 plot^{-1}	ROCHE, DIXON and PATE, 1998 BELL, 1999
Laxmannia spp.	WA	SA/ssb	0 plot^{-1}	17 ± 8.33 plot^{-1}	ROKICH, DIXON, SIVASITHAMPARAM and MENEY, 2002
Thysanotus fastigiatus	WA/rm WA/jf	SA/ssb	0.00 m^{-2}	0.94 m^{-2}	GRANT and KOCH, 1997 ROCHE, KOCH and DIXON, 1997
Thysanotus manglesianus	WA	SA/rm	0 plot^{-1}	1 ± 0.58 plot^{-1}	ROKICH, DIXON, SIVASITHAMPARAM and MENEY, 2002
Thysanotus multiflorus	WA/jf WA/rm	SA SW SA/ssb SW/ssb SA/bcs PI	0 0 0.00 m^{-2} 24.00 plot^{-1} 15.5 plot^{-1} 1.83 4m^{-2}	~ 30 23.9 ± 2 4.69 m^{-2} 42.83 plot^{-1} 37.33 plot^{-1} 3.72 4m^{-2} 8.22 4m^{-2} 25.05 4m^{-2}	DIXON, ROCHE and PATE, 1995 GRANT and KOCH, 1997 ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999
CENTROLEPIDACEAE					
Centrolepis aristata	Aus	SW/ssb			ENRIGHT and KINTRUP, 2001
Centrolepis strigosa	Aus	SW/ssb			ENRIGHT and KINTRUP, 2001

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
COLCHICACEAE					
Burchardia umbellata	WA/jf WA/rm	CH SA/bcs SW PI SA	9 5.00 plot^{-1} 26.89 4m^{-2} ~ 20 24.1 ± 1.5	35 11.17 plot^{-1} 60.83 4m^{-2} 82.00 4m^{-2} 101.00 4m^{-2} ~ 35 41.5 ± 1.2	BELL, VLAHOS and WATSON, 1987 DIXON, ROCHE and PATE, 1995 ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999
CYPERACEAE					
Cyathochaeta avenacea	WA/rm	SA/bcs	1.75 plot^{-1}	4.50 plot^{-1}	ROCHE, KOCH and DIXON, 1997
Gahnia lanigera	Aus	SA	0.6 ± 0.3	14.5 ± 1.9	ROCHE, DIXON and PATE, 1997 BELL, 1999
Isolepis marginata	Aus	SW/ssb			ENRIGHT and KINTRUP, 2001
DASYPOGONACEAE					
Acanthocarpus preissii	Aus	SA/storage	0	13.3 ± 3.5	ROCHE, DIXON and PATE, 1997 BELL, 1999
Lomandra multiflora	Aus	SA	1.6 ± 0.7	12.1 ± 2.2	ROCHE, DIXON and PATE, 1997 BELL, 1999
Lomandra sp.	WA/rm	SW/ssb	1.67 plot^{-1}	3.83 plot^{-1}	
HAEMODORACEAE					
Anigozanthos bicolor	WA	SA	< 4	~ 8	DIXON, ROCHE and PATE, 1995 BELL, 1999
Anigozanthos humilis	WA	SA	~ 8	~ 20	DIXON, ROCHE and PATE, 1995 BELL, 1999
Anigozanthos manglesii	WA	SA SW	~ 8 ~ 15 6.1 ± 0.5	~ 30 ~ 40 28.8 ± 1.3	DIXON, ROCHE and PATE, 1995 ROCHE, DIXON and PATE, 1997 BELL, 1999 TIEU, DIXON, MENEY, SIVASITHAMPARAM and BARRETT, 2001
Anigozanthos rufus	Aus	SA	7.9 ± 0.5	20.3 ± 1.8	ROCHE, DIXON and PATE, 1997 BELL, 1999
Blancoa canescens	Aus	SA	0	47.7 ± 10	ROCHE, DIXON and PATE, 1997 BELL, 1999
Conostylis aculeata	WA	SA SA/rm SA/ssb	10.7 ± 0.8 1 ± 1 plot^{-1} 0 plot^{-1}	30.9 ± 2 3.0 ± 0 plot^{-1} 16 ± 5.69 plot^{-1}	ROCHE, DIXON and PATE, 1997 BELL, 1999 ROKICH, DIXON, SIVASITHAMPARAM and MENEY, 2002
Conostylis candicans	WA	SA/SW	< 5	± 20	LLOYD, DIXON and SIVASITHAMPARAM, 2000
Conostylis neocymosa	WA	SA	~ 15	~ 95	DIXON, ROCHE and PATE, 1995 BELL, 1999
Conostylis serrulata	WA/rm	SA/bcs	0.25 plot^{-1}	1.25 plot^{-1}	ROCHE, KOCH and DIXON, 1997
Conostylis setigera	WA	SW PI	± 10	± 60	TIEU, DIXON, SIVASITHAMPARAM, PLUMMER and SIELER, 1999
Conostylis setosa	WA WA/jf	SA SA/ssb	< 5 0.00 m^{-2}	~ 50 1.67 m^{-2}	DIXON, ROCHE and PATE, 1995 ROCHE, KOCH and DIXON, 1997 BELL, 1999
Haemodorum simplex	Aus	SA	50.4 ± 3	68.7 ± 3	ROCHE, DIXON and PATE, 1997 BELL, 1999
Wachendorfia paniculata	CFR	S	60	78	BROWN and BOTHA, 2002, 2004
Wachendorfia thyrsiflora	CFR	S	15	54	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
IRIDACEAE					
Aristea africana	CFR	S	23	46	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Aristea racemosa	CFR	S	68	90	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Orthrosanthus laxus	Aus	SA	5.7 ± 0.8	20.7 ± 1.6	ROCHE, DIXON and PATE, 1997 BELL, 1999
Patersonia glabrata	Aus	SA	0	5.2 ± 1.3	ROCHE, DIXON and PATE, 1997 BELL, 1999
Patersonia occidentalis	WA	SA	0 0.4 ± 0.2	~ 25 12.4 ± 1.3	DIXON, ROCHE and PATE, 1995 ROCHE, DIXON and PATE, 1997 BELL, 1999
Patersonia sp. aff. *fragilis*	Aus	SA	11.8 ± 2.3	56.5 ± 4.4	ROCHE, DIXON and PATE, 1997
Patersonia umbrosa	Aus	SA	0	1.4 ± 0.3	ROCHE, DIXON and PATE, 1997
Sisyrinchium sp.	NSW	SA/ssb	13 ± 7 m^{-2}	874 ± 344 m^{-2}	READ, BELLAIRS, MULLIGAN and LAMB, 2000
ORCHIDACEAE					
Monadenia bracteata (syn. *Disa bracteata*)	WA/rm	SA/ssb			GRANT and KOCH, 1997
PHORMIACEAE					
Dianella brevicaulis	Aus	SA	0	35.7 ± 5.4	ROCHE, DIXON and PATE, 1997 BELL, 1999
Dianella revoluta	Aus	SA	6.3 ± 0.9	34.7 ± 3.5	ROCHE, DIXON and PATE, 1997
Dianella sp. aff. *longifolia*	Aus	SA	33.3 ± 2.4	59.7 ± 3.7	ROCHE, DIXON and PATE, 1997
Dianella tarda	Aus	SA	21.4 ± 4.3	53.7 ± 9	ROCHE, DIXON and PATE, 1997 BELL, 1999
POACEAE					
Achnatherum hymenoides	US	SA			BLANK and YOUNG, 1998
Achnatherum occidentalis	US	SA			BLANK and YOUNG, 1998
Achnatherum thurberianum	US	SA			BLANK and YOUNG, 1998
Alopecurus myosuroides		SW	45 ± 9	88 ± 12	ADKINS and PETERS, 2001 ADKINS, PETERS, PATERSON and NAVIE, 2003
Amphipogon amphipogonoides	Aus WA/jf WA/rm	SA SA/ssb SW/ssb SA/bcs	19 ± 1.5 0.00 m^{-2} 2.17 $plot^{-1}$ 3.33 $plot^{-1}$	53.2 ± 4.5 2.19 m^{-2} 4.67 $plot^{-1}$ 10.17 $plot^{-1}$	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999
Aristida ramosa		SW			ADKINS, PETERS, PATERSON and NAVIE, 2003
Austrodanthonia tenuior	Aus	SW			CLARKE and FRENCH, 2005
Austrostipa compressa	WA	SW	~ 25	~ 80	SMITH, BELL and LONERAGAN, 1999
Avena fatua		SW	0	92	ADKINS and PETERS, 2001 ADKINS, PETERS, PATERSON and NAVIE, 2003 KĘPCZYŃSKI, BIAŁECKA, LIGHT and VAN STADEN, 2006
Avena sterilis ssp. *ludoviciana*	NSW	SW	< 10 12 ± 6	> 80 93 ± 3	ADKINS, DAVIDSON, MATTHEW, NAVIE, WILLS, TAYLOR and BELLAIRS, 2000 ADKINS and PETERS, 2001
Cenchrus ciliaris		SW			ADKINS, PETERS, PATERSON and NAVIE, 2003

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Chloris ventricosa	NSW	SW	89.6	97	READ and BELLAIRS, 1999
Dactylis glomerata	MB	SA CE			PÉREZ-FERNÁNDEZ and RODRÍGUEZ-ECHEVERRÍA 2003
Dichanthium sericeum	NSW	SW	93.0	98.3	READ and BELLAIRS, 1999
Digitaria diffusa	NSW	SA/ssb	11 ± 4 m^{-2}	37 ± 10 m^{-2}	READ, BELLAIRS, MULLIGAN and LAMB, 2000
Digitaria ramularis	NSW	SA/ssb	0 ± 0 m^{-2}	8 ± 4 m^{-2}	READ, BELLAIRS, MULLIGAN and LAMB, 2000
Eragrostic curvula	Aus	SW			CLARKE and FRENCH, 2005
Eragrostis benthamii	Aus	SW			CLARKE and FRENCH, 2005
Eragrostis cilianensis	NSW	SA/ssb	14 ± 3 m^{-2}	64 ± 17 m^{-2}	READ, BELLAIRS, MULLIGAN and LAMB, 2000
Eragrostis leptostachya	Aus	SA/ssb SW	19 ± 9 m^{-2}	73 ± 30 m^{-2}	READ, BELLAIRS, MULLIGAN and LAMB, 2000 CLARKE and FRENCH, 2005
Eragrostis sororia	NSW	SA/ssb	7 ± 2 m^{-2}	42 ± 15 m^{-2}	READ, BELLAIRS, MULLIGAN and LAMB, 2000
Festuca megalura	SCal	CH	85	97	KEELEY, MORTON, PEDROSA and TROTTER, 1985
Hesperostipa comata	US	SA			BLANK and YOUNG, 1998
Heteropogon contortus		SW			ADKINS, PETERS, PATERSON and NAVIE, 2003
Melinis minutiflora	QL	SA/ssb			ADKINS, DAVIDSON, MATTHEW, NAVIE, WILLS, TAYLOR and BELLAIRS, 2000
Neurachne alopecuroidea	WA	SA	~ 60	~ 100	DIXON, ROCHE and PATE, 1995 BELL, 1999
Oryza sativa		SWcls	6 ± 3	78 ± 4	DOHERTY and COHN, 2000
Panicum decompositum	NSW	SW	7.7	63.1	READ and BELLAIRS, 1999
Panicum effusum	NSW	SW SA/ssb	0 0 ± 0 m^{-2}	16.7 14 ± 4 m^{-2}	READ and BELLAIRS, 1999 READ, BELLAIRS, MULLIGAN and LAMB, 2000 CLARKE and FRENCH, 2005
Panicum maximum	QL	SA/ssb			ADKINS, DAVIDSON, MATTHEW, NAVIE, WILLS, TAYLOR and BELLAIRS, 2000
Panicum simile	Aus	SW			CLARKE and FRENCH, 2005
Paspalidium distans	NSW	SW SA/ssb	17.5 32 ± 9 m^{-2}	30.5 102 ± 30 m^{-2}	READ and BELLAIRS, 1999 READ, BELLAIRS, MULLIGAN and LAMB, 2000
Pentaschistis colorata	CFR	S	0	24 ± 7	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Phalaris paradoxa	QL	SW	< 30 50 ± 5	> 80 100 ± 0	ADKINS, DAVIDSON, MATTHEW, NAVIE, WILLS, TAYLOR and BELLAIRS, 2000 ADKINS and PETERS, 2001 ADKINS, PETERS, PATERSON and NAVIE, 2003
Poa labillardieri	NSW	SW	10	23.5	READ and BELLAIRS, 1999
Pseudopentameris macrantha	CFR	S	5	26	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Sorghum halepense		SW	12 ± 3	100 ± 0	ADKINS and PETERS, 2001 ADKINS, PETERS, PATERSON and NAVIE, 2003

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Stipa compressa	WA	SA	55 ± 16 plot^{-1}	441 ± 53 plot^{-1}	ROCHE, DIXON and PATE, 1998 BELL, 1999
Stipa scabra subsp. *falcata*	NSW	SW	14.0	34.1	READ and BELLAIRS, 1999
Tetrarrhena laevis	WA/rm Aus	SA/bcs SA	9.5 plot^{-1} 1.3 ± 1.3	15.75 plot^{-1} 17.7 ± 2.6	ROCHE, KOCH and DIXON, 1997 ROCHE, DIXON and PATE, 1997
Themeda triandra	NSW	SW	75.2	86.6	READ and BELLAIRS, 1999
Themeda triandra	CFR	SA, SW	6.0 ± 2.3	35.8 ± 3.1	BAXTER, VAN STADEN, GRANGER and BROWN, 1994 BAXTER and VAN STADEN, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Zea mays	SA	SA			MODI, 2002 SPARG, KULKARNI and VAN STADEN, 2006
RESTIONACEAE					
Askidiosperma andreaeanum	CFR	SA	6 ± 4	42 ± 13	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Askidiosperma paniculatum	CFR	S	53	67	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Cannomois virgata	CFR	SA S CH	2 ± 5 12 ± 30	18 ± 13 42 ± 70	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 NEWTON, BOND and FARRANT, 2006
Chondropetalum ebracteatum	CFR	S	66	85	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Chondropetalum hookerianum	CFR	SA S	1 ± 2 2	61 ± 24 10	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Chondropetalum mucronatum	CFR	SA S	4 ± 4 14 35	81 ± 9 86 90	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Chondropetalum tectorum	CFR	SA S	1 ± 2 g^{-1} 0.1	21 ± 17 g^{-1} 2.1	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Dovea macrocarpa	CFR	SA	2 ± 4	77 ± 8	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Elegia capensis	CFR	S	0.3	1.0	BROWN and BOTHA, 1995, 2004

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Elegia cuspidata	CFR	SA	14 ± 7 g^{-1} 2.4	30 ± 10 g^{-1} 5.2	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Elegia equisetacea	CFR	S	3	14	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Elegia fenestrata	CFR	SA	164 ± 30 g^{-1} 11.3	306 ± 25 g^{-1} 21.1	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Elegia filacea	CFR	S	16	32	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Elegia grandis	CFR	S	24	43	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Elegia persistens	CFR	S	1	15	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Elegia spathacea	CFR	S	13	54	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Georgiella hexandra	Aus	SA	0	6.4 ± 0.7	ROCHE, DIXON and PATE, 1997 BELL, 1999
Hypodiscus sp.	CFR	S	0	7	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Ischyrolepis ocreata	CFR	S	10	47	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Ischyrolepis sieberi	CFR	SW SA	80 ± 4 1 ± 2	92 ± 5 69 ± 11	BROWN, 1993a BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Ischyrolepis subverticillata	CFR	SA	5 ± 5	64 ± 8	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Mastersiella digitata	CFR	S			BROWN and BOTHA, 2002
Restio bifarius	CFR	S	30	45	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Restio brachiatus	CFR	S	53	76	BROWN and BOTHA, 1995, 2004
Restio dispar	CFR	S	12 26 80	61 37 88	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Restio festuciformis	CFR	SA	281 ± 25 g^{-1} 9.0	412 ± 120 g^{-1} 13.2	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Restio praeacutus	CFR	S			BROWN and BOTHA, 1995

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Restio similis	CFR	SW SA	82 ± 3 13 ± 21	94 ± 4 44 ± 18	BROWN, 1993a BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Restio tetragonus	CFR	SA	2 ± 2	97 ± 4	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Restio triticeus	CFR	SA	37 ± 15	94 ± 8	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Rhodocoma arida	CFR	S	19	54	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Rhodocoma capensis	CFR	SA	10 ± 22 g^{-1} 0.2	2410 ± 315 g^{-1} 50.6	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Rhodocoma fruticosa	CFR	S	68	97	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Rhodocoma gigantea	CFR	SA PI	1 ± 2 23 ± 6	74 ± 25 79 ± 11	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, PROSCH and BOTHA, 1998 BROWN and VAN STADEN, 1998 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Staberoha aemula	CFR	SA	1 ± 2	62 ± 8	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Staberoha banksii	CFR	S	4	42	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Staberoha cernua	CFR	SA	1 ± 2 1 ± 2	26 ± 12 43 ± 8	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Staberoha distachyos	CFR	S	6 ± 2	32 ± 15	BROWN, 1993a BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Staberoha vaginata	CFR	SA	1 ± 2	8 ± 4	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Thamnochortus bachmannii	CFR	SA	1 ± 2 g^{-1} 0.1	61 ± 12 g^{-1} 7.9	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Thamnochortus cinereus	CFR	SA	6 ± 5 g^{-1} 1.1	107 ± 44 g^{-1} 20.3	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Thamnochortus ebracteatum	CFR	S			BROWN and BOTHA, 1995
Thamnochortus insignis	CFR	S	10	28	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Thamnochortus pellucidus	CFR	SA	1 ± 1 1 ± 2 g^{-1} 0.3	24 ± 7 27 ± 14 g^{-1} 7.9	BROWN, 1993a BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Thamnochortus platypteris	CFR	S	1	4	BROWN and BOTHA, 2004
Thamnochortus punctatus	CFR	SA	1 ± 1 g^{-1} 0.05	38 ± 13 g^{-1} 2.2	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Thamnochortus spicigerus	CFR	SA	1 ± 2 g^{-1} 0.4	12 ± 3 g^{-1} 3.7	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Thamnochortus sporadicus	CFR	S	1 ± 1	4 ± 2	BROWN, JAMIESON and BOTHA, 1994 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Willdenowia incurvata	CFR	S	2	6	BROWN and BOTHA, 2002, 2004
XANTHORRHOEACEAE					
Xanthorrhoea sp.	WA/rm	SA/bcs	73.17 $plot^{-1}$	100.83 $plot^{-1}$	ROCHE, KOCH and DIXON, 1997
Dicotyledonae					
ANACARDIACEAE					
Rhus integrifolia	SCal	CH	43	88	KEELEY, 1987
Rhus trilobata	SCal	CH	38 17	65 28	KEELEY, 1987
Toxicodendron diversifolia	SCal	CH	10	20	KEELEY, 1987
APIACEAE					
Actinotus helianthi	Aus	SA	15 ± 0.9	56.2 ± 3.4	ROCHE, DIXON and PATE, 1997 BELL, 1999

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Actinotus leucocephalus	WA	SW/PI SW	0 ~50	~ 10 ~ 95	TIEU, DIXON, SIVASITHAMPARAM, PLUMMER and SIELER, 1999 BAKER, STEADMAN, PLUMMER, MERRITT and DIXON, 2005a
Hydrocotyle callicarpa	WA/jf	SA/ssb SW/ssb	0.31 m^{-2}	20.94 m^{-2}	ROCHE, KOCH and DIXON, 1997 ENRIGHT and KINTRUP, 2001
Platysace compressa	WA/jf WA/rm	SA/ssb SA/ssb	0.83 m^{-2} 0.36 m^{-2}	27.08 m^{-2} 1.35 m^{-2}	GRANT and KOCH, 1997 ROCHE, KOCH and DIXON, 1997
Trachymene pilosa	WA/rm	SW/ssb	0.33 $plot^{-1}$	2.33 $plot^{-1}$	GRANT and KOCH, 1997 ROCHE, KOCH and DIXON, 1997
Xanthosia candida	WA/jf	SA/ssb	0.31 m^{-2}	31.35 m^{-2}	ROCHE, KOCH and DIXON, 1997
Xanthosia heugelii	WA/jf WA.rm	SA/ssb SW/ssb	0 0.00 m^{-2} 4.30 $plot^{-1}$	13.9 ± 1.9 4.17 m^{-2} 11.17 $plot^{-1}$	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999
ASTERACEAE					
Agoseris heterophylla	SCal	CH	62	92	KEELEY and KEELEY, 1987
Angianthus tomentosus	WA	SC SW			MERRITT, KRISTIANSEN, FLEMATTI, TURNER, GHISALBERTI, TRENGOVE and DIXON, 2006
Arctotis stoechadifolia	CFR	S	4	23	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Artemisia californica	SCal	CH	0	62	KEELEY, 1987
Chaenactis artemisiifolia	SCal	CH S	39 27	53 84	KEELEY, MORTON, PEDROSA and TROTTER, 1985 KEELEY and FOTHERINGHAM, 1998b
Corymbium glabrum var. *glabrum*	CFR	S	3	5	BROWN and BOTHA, 2002 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Corymbium laxum subsp. *bolusii*	CFR	S	14	22	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Dimorphotheca nudicaulis (syn. *Castalis nudicaulis*)	CFR	S	4	9	BROWN and BOTHA, 2004
Dittrichia viscosa	MB	SA CE			PÉREZ-FERNÁNDEZ and RODRÍGUEZ-ECHEVERRÍA 2003
Edmondia sesamoides	CFR	S	11 ± 1	98 ± 1	BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Eriocephalus africanus	CFR	S			BROWN and BOTHA, 2002 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Eriophyllum confertiflorum	SCal	CH	10 4 11	57 52 61	KEELEY and NITZBERG, 1984 KEELEY, MORTON, PEDROSA and TROTTER, 1985 KEELEY and PIZZORNO, 1986
Euryops linearis	CFR	S	31	73	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Euryops speciosissimus	CFR	S	24	64	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Euryops virgineus	CFR	S	6	31	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Felicia aethiopica subsp. *aethiopica*	CFR	S	18	30	BROWN and BOTHA, 2004
Felicia heterophylla	CFR	S	22	46	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Gnaphalium californica	SCal	CH	46	79	KEELEY and KEELEY, 1987
Gnaphalium sphaericum	WA/rm	SA/ssb			GRANT and KOCH, 1997
Gnephosis tenuissima	WA	SC SW			MERRITT, KRISTIANSEN, FLEMATTI, TURNER, GHISALBERTI, TRENGOVE and DIXON, 2006
Helichrysum foetidum	CFR	S	12 ± 1	64 ± 1	BROWN and BOTHA, 1995 BROWN and BOTHA, 2002 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Helichrysum patulum (syn. *H. crispum*)	CFR	S	24 ± 1	98 ± 1	BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Helichrysum tinctum	CFR	S			BROWN and BOTHA, 2002 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Hyalosperma cotula	WA/rm	SA/bcs	33.00 plot^{-1}	36.00 plot^{-1}	ROCHE, KOCH and DIXON, 1997
Ixodia achillaeoides	Aus	SW/ssb			ENRIGHT and KINTRUP, 2001
Lactuca sativa					See Chapter 3
Malacothrix clevelandii	SCal	CH	9	35	KEELEY and KEELEY, 1987
Metalasia densa (syn. *Metalasia muricata*)	CFR	SA SW	1 ± 1 2 ± 1	14 ± 3 14 ± 5	BROWN, 1993a BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Myriocephalus guerinae	WA	SC SW			MERRITT, KRISTIANSEN, FLEMATTI, TURNER, GHISALBERTI, TRENGOVE and DIXON, 2006
Oedera capensis	CFR	S	5	20	BROWN and BOTHA, 2002, 2004
Othonna bulbosa	CFR	S			BROWN and BOTHA, 2002
Othonna parviflora	CFR	S			BROWN and BOTHA, 2002 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Othonna quinquedentata	CFR	SA S	44 ± 7 24 ± 5	63 ± 7 34 ± 7	BROWN, 1993a BROWN and BOTHA, 1995, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Parthenium argentatum	USA	SW	± 50%	70-80%	BEKAARDT, PIETERSE, COETZEE and AGENBAG, 2004
Phaenocoma prolifera	CFR	S	59 ± 3	97 ± 1	BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Podolepis canescens	WA	SC SW			MERRITT, KRISTIANSEN, FLEMATTI, TURNER, GHISALBERTI, TRENGOVE and DIXON, 2006
Rafinesquia californica	SCal	CH	4	55	KEELEY and KEELEY, 1987
Rhodanthe citrina	WA	SC			MERRITT, KRISTIANSEN, FLEMATTI, TURNER, GHISALBERTI, TRENGOVE and DIXON, 2006

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Schoenia filifolia subsp. *subulifolia*	WA	SW (dark)	0	~ 40	PLUMMER, ROGERS, TURNER and BELL, 2001
Senecio grandiflorus	CFR	SA S	56 ± 19	79 ± 6	BROWN, 1993a BROWN and BOTHA, 1995 BROWN and BOTHA, 2002
Senecio jacobea	MB	SA CE			PÉREZ-FERNÁNDEZ and RODRÍGUEZ-ECHEVERRÍA 2003
Senecio rigidus	CFR	S	80	90	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Senecio umbellatus	CFR	S	56 ± 19	79 ± 6	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Stephanomeria virgata	SCal	CH	45	56	KEELEY and KEELEY, 1987
Syncarpha eximia (syn. *Helipterum eximium*)	CFR	SA SW	19 ± 8 29 ± 9	80 ± 10 87 ± 8	BROWN, 1993a BROWN and BOTHA, 1995, 2004
Syncarpha speciosissima	CFR	S	17 ± 3	30 ± 1	BROWN and BOTHA, 1995, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Syncarpha vestita (syn. *Helichrysum vestitum*)	CFR	SA SW CE PI	2 ± 2 8 ± 3 5 ± 4 6 ± 4 8 ± 4 2 ± 2	78 ± 14 81 ± 3 77 ± 16 94 ± 4 100 98 ± 2	BROWN, 1993a, 1993b BROWN and BOTHA, 1995, 2002, 2004 BROWN, PROSCH and BOTHA, 1998 BROWN and VAN STADEN, 1998 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Ursinia paleacea	CFR	S			BROWN and BOTHA, 2002 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Vellereophyton dealbatum	WA/rm	SA/ssb			GRANT and KOCH, 1997
BETULACEAE					
Alnus glutinosa	MB	SA			CROSTI, LADD, DIXON and PIOTTO, 2006
BORAGINACEAE					
Cryptantha clevelandi	SCal	S			KEELEY and FOTHERINGHAM, 1998b
Cryptantha intermedia	SCal	CH	55	74	KEELEY and KEELEY, 1987
Cryptantha micrantha	SCal	S			KEELEY and FOTHERINGHAM, 1998b
Cryptantha muricata	SCal	CH	24	67	KEELEY, MORTON, PEDROSA and TROTTER, 1985
Galium aparine		SW	5 ± 1	27 ± 3	ADKINS and PETERS, 2001
Galium aparine		SW			ADKINS, PETERS, PATERSON and NAVIE, 2003
BRASSICACEAE					
Brassica napus cv. Apex			< 10	~ 60	THORNTON, THOMAS and PETERS, 1999
Caulanthus heterophyllus	SCal	S			KEELEY and FOTHERINGHAM, 1998b
Heliophila pinnata	CFR	S			BROWN and BOTHA, 2002
Heliophila sp.	CFR	S			BROWN and BOTHA, 2002 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Lepidium nitidum	SCal	CH	2	22	KEELEY and KEELEY, 1987
Streptanthus heterophyllus	SCal	CH	1	25	KEELEY and KEELEY, 1987

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
BRUNIACEAE					
Audouinia capitata	CFR	SA/SW	3.6	14.3/17.9	DE LANGE and BOUCHER, 1990, 1993a, 1993b BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Berzelia lanuginosa	CFR	S			BROWN and BOTHA, 1995
Brunia albiflora	CFR	S	1	9	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Brunia laevis	CFR	S	1	6	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
CAMPANULACEAE					
Lobelia coronopifolia	CFR	S	1	5	BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Lobelia linearis	CFR	S	2	8	BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Lobelia sp.	CFR	S	0	9	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Monopsis lutea	CFR	S	10	35	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Roella ciliata	CFR	S	32	92	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Roella triflora	CFR	S	5	50	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Wahlenbergia cernua	CFR	S S	0	45 ± 6	BROWN and BOTHA, 2002 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Wahlenbergia gracilenta	Aus	SW/ssb			ENRIGHT and KINTRUP, 2001
Wahlenbergia gracilis	NSW	SA/ssb	275 ± 36 m^{-2}	545 ± 126 m^{-2}	READ, BELLAIRS, MULLIGAN and LAMB, 2000
CARYOPHYLLACEAE					
Paronychia sp.	NSW	SA/ssb	0 ± 0 m^{-2}	6 ± 3 m^{-2}	READ, BELLAIRS, MULLIGAN and LAMB, 2000
Silene clandestina	CFR	S			BROWN and BOTHA, 2002
Silene cretica	CFR	S	2 ± 1	15 ± 2	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Silene multinervia	SCal	CH S	6	44	KEELEY and KEELEY, 1987 KEELEY and FOTHERINGHAM, 1998b
CASUARINACEAE					
Allocasuarina fraseriana	WA/rm	SA/bcs	37.17 $plot^{-1}$	58.17 $plot^{-1}$	ROCHE, KOCH and DIXON, 1997
CISTACEAE					
Cistus crispus	MB	SA CE			PÉREZ-FERNÁNDEZ and RODRÍGUEZ-ECHEVERRÍA 2003
Cistus incanus	MB	SA			CROSTI, LADD, DIXON and PIOTTO, 2006

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Cistus ladanifer	MB	SA CE			PÉREZ-FERNÁNDEZ and RODRÍGUEZ-ECHEVERRÍA 2003
Cistus monspeliensis	MB	SA			PÉREZ-FERNÁNDEZ and RODRÍGUEZ-ECHEVERRÍA 2003
Cistus salvilifloius	MB	SA CE			PÉREZ-FERNÁNDEZ and RODRÍGUEZ-ECHEVERRÍA 2003
CRASSULACEAE					
Crassula capensis	CFR	S			BROWN and BOTHA, 2002 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Crassula closiana	Aus	SW/ssb			ENRIGHT and KINTRUP, 2001
DILLENIACEAE					
Hibbertia amplexicaulis	WA/rm	SA SW SW/ssb SA/bcs	0 1.3 ± 0.3 plot^{-1} 3.83 plot^{-1} 4.00 plot^{-1}	~ 5 28 ± 5 plot^{-1} 7.33 plot^{-1} 7.5 plot^{-1}	DIXON, ROCHE and PATE, 1995 ROCHE, DIXON and PATE, 1998 ROCHE, KOCH and DIXON, 1997 BELL, 1999 ADKINS, PETERS, PATERSON and NAVIE, 2003
Hibbertia commutata		SA SW	0	~ 6	TIEU, DIXON, MENEY and SIVASITHAMPARAM, 2001b ADKINS, PETERS, PATERSON and NAVIE, 2003
Hibbertia lasiopus	WA	SA	0 0.3 ± 0.3	~ 4 11.8 ± 1.2	DIXON, ROCHE and PATE, 1995 ROCHE, DIXON and PATE, 1997 BELL, 1999
Hibbertia ovata	Aus	SA	0.5 ± 0.5	6.1 ± 1	ROCHE, DIXON and PATE, 1997 BELL, 1999
Hibbertia quadricolor	WA	SA	0 0	~ 4 5.7 ± 1.3	DIXON, ROCHE and PATE, 1995 ROCHE, DIXON and PATE, 1997 BELL, 1999
Hibbertia racemosa	WA	SA/ssb SA/rm	1.67 ± 0.33 plot^{-1} 23.33 ± 0.3 plot^{-1}	133 ± 32.9 plot^{-1} 45.37 ± 1.2 plot^{-1}	ROKICH, DIXON, SIVASITHAMPARAM and MENEY, 2002
Hibbertia riparia	Aus	SA	1.1 ± 0.3	17.1 ± 0.9	ROCHE, DIXON and PATE, 1997 BELL, 1999
Hibbertia sericea	Aus	SA	0	14 ± 4.2	ROCHE, DIXON and PATE, 1997 BELL, 1999
Hibbertia serrata	Aus	SA	0	1.9 ± 0.6	ROCHE, DIXON and PATE, 1997 BELL, 1999
Hibbertia subvaginata	WA	SA/rm	2.33 ± 0.88 plot^{-1}	7.33 ± 1.45 plot^{-1}	ROKICH, DIXON, SIVASITHAMPARAM and MENEY, 2002
DROSERACEAE					
Drosera glanduligera	Aus	SW/ssb			ENRIGHT and KINTRUP, 2001
EPACRIDACEAE					
Acrotriche patula	Aus	SA	0	0.7 ± 0.3	ROCHE, DIXON and PATE, 1997 BELL, 1999
Andersonia involucrata	Aus	SA	5.2 ± 0.9	20.9 ± 1.8	ROCHE, DIXON and PATE, 1997 BELL, 1999
Andersonia latiflora	Aus	SA	19.9 ± 2.7	55.3 ± 5.5	ROCHE, DIXON and PATE, 1997 BELL, 1999
Andersonia lehmanniana	WA	SA	~ 4	~ 16	DIXON, ROCHE and PATE, 1995 BELL, 1999
Astroloma macrocalyx	WA	SA/rm	10 ± 4.73 plot^{-1}	68.67 ± 8.0 plot^{-1}	ROKICH, DIXON, SIVASITHAMPARAM and MENEY, 2002

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Epacris apsleyensis	Aus	SW/heat	4.3 ± 0.6	72.7 ± 2.5	GILMOUR, CROWDEN and KOUTOULIS, 2000
Epacris impressa	Aus	SW/ssb			ENRIGHT and KINTRUP, 2001
Epacris lanuginosa	Aus	SW/heat	0.7 ± 0.4	42.7 ± 5.2	GILMOUR, CROWDEN and KOUTOULIS, 2000
Epacris obtusifolia	Aus	SA SW/heat	7.8 ± 1.9 3.0 ± 0.9	20.8 ± 2.5 64.7 ± 5.7	ROCHE, DIXON and PATE, 1997 BELL, 1999 GILMOUR, CROWDEN and KOUTOULIS, 2000
Epacris purpurascens	Aus	SW/heat	3.3 ± 0.7	75.0 ± 4.6	GILMOUR, CROWDEN and KOUTOULIS, 2000
Epacris stuartii	Aus	SW/SS			KEITH, 1997
Epacris tasmanica	Aus	SW/heat	5.3 ± 1.3	49.0 ± 7.1	GILMOUR, CROWDEN and KOUTOULIS, 2000
Leucopogon capitellatus	Aus	SA	0	19.4 ± 2.5	ROCHE, DIXON and PATE, 1997 BELL, 1999
Leucopogon conostephioides	WA	SA	41 ± 4 plot^{-1}	193 ± 20 plot^{-1}	ROCHE, DIXON and PATE, 1998 BELL, 1999
Leucopogon glacialis	Aus	SW/ssb			ENRIGHT and KINTRUP, 2001
Leucopogon nutans	Aus WA/rm	SA SW/ssb SA/bcs	0 1.33 plot^{-1} 1.17 plot^{-1}	9.5 ± 3 3.83 plot^{-1} 3.17 plot^{-1}	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999
Leucopogon sp.	WA	SA/ssb SA/rm	0 plot^{-1} 13.67 ± 4.3 plot^{-1}	32.7 ± 21.3 plot^{-1} 73.33 ± 5.8 plot^{-1}	ROKICH, DIXON, SIVASITHAMPARAM and MENEY, 2002
Leucopogon sp.	WA	SW/fs	1 plot^{-1}	65 plot^{-1}	LLOYD, DIXON and SIVASITHAMPARAM, 2000
Sphenotoma capitatum	WA	SA	0	~ 45	DIXON, ROCHE and PATE, 1995 BELL, 1999
ERICACEAE					
Arctostaphylos glandulosa	SCal	CH	0 0	5 8	KEELEY, 1987
Arctostaphylos patula	SCal	CH	6	17	KEELEY, 1987
Calluna vulgaris	UK	SW/GA		10-fold	THOMAS and DAVIES, 2002
Erica baccans	CFR	S	9	37	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica caffra	CFR	S			BROWN and BOTHA, 1995 BROWN and BOTHA, 2002 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica canaliculata	CFR	SA	528 ± 280 g^{-1}	3540 ± 860 g^{-1}	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica capensis	CFR	SA	56 ± 16 g^{-1}	104 ± 26 g^{-1}	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica capitata	CFR	S			BROWN and BOTHA, 1995
Erica cerinthoides	CFR	S			BROWN and BOTHA, 1995

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Erica clavisepala	CFR	SA	4 ± 9 g^{-1}	324 ± 110 g^{-1}	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica curvirostris	CFR	SA	470 ± 132 g^{-1}	990 ± 313 g^{-1}	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica deflexa	CFR	SA	168 ± 48 g^{-1}	266 ± 66 g^{-1}	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica diaphana	CFR	S			BROWN and BOTHA, 1995 BROWN and BOTHA, 2002
		S			BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica dilatata	CFR	S			BROWN and BOTHA, 1995
Erica discolor	CFR	SA	162 ± 48 g^{-1} 2.2 ± 0.7	266 ± 49 g^{-1} 3.6 ± 0.7	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica ericoides (syn. *Blaeria ericoides*)	CFR	SA	1552 ± 220 g^{-1}	2524 ± 320 g^{-1}	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica formosa	CFR	SA	1928 ± 671 g^{-1} 3.8 ± 1.3	4374 ± 336 g^{-1} 8.6 ± 0.7	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica glauca var. *glauca*	CFR	SA	14 ± 9 g^{-1} 0.16 ± 0.1	1060 ± 180 g^{-1} 11.8 ± 2.0	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica glauca var. *elegans*	CFR	S			BROWN and BOTHA, 1995
Erica glomiflora	CFR	SA, SW	734 ± 185 g^{-1} 24 ± 18 g^{-1} 1480 ± 270 g^{-1} 0.15 ± 0.1	1765 ± 200 g^{-1} 402 ± 63 g^{-1} 2950 ± 430 g^{-1} 2.6 ± 0.4	BROWN, 1993a BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica grata	CFR	SA	404 ± 76 g^{-1}	4250 ± 680 g^{-1}	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Erica hebecalyx	CFR	SA SW	614 ± 125 g^{-1} 1370 ± 75 g^{-1}	1766 ± 235 g^{-1} 2660 ± 285 g^{-1}	BROWN, 1993a BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica hirtiflora	CFR	S S			BROWN and BOTHA, 1995 BROWN and BOTHA, 2002 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica junonia var. *minor*	CFR	S			BROWN and BOTHA, 1995
Erica lateralis	CFR	SA	698 ± 111 g^{-1} 1.7 ± 0.3	4330 ± 1360 g^{-1} 10.8 ± 3.4	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica latiflora	CFR	SA	16 ± 4 g^{-1}	416 ± 16 g^{-1}	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995 BROWN and BOTHA, 2002
Erica longifolia	CFR	SW	350 ± 105 g^{-1}	2580 ± 250 g^{-1}	BROWN, 1993a BROWN and BOTHA, 1995, 2002
Erica nudiflora	CFR	SA	2 ± 4 g^{-1}	20 ± 10 g^{-1}	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica oatesii	CFR	SA	408 ± 256 g^{-1}	1604 ± 928 g^{-1}	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica perlata	CFR	SA	10800 ± 4580 g^{-1}	33940 ± 5440 g^{-1}	BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica phylicifolia	CFR	SA	26 ± 9 g^{-1}	296 ± 18 g^{-1}	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica pinea	CFR	SA	184 ± 47 g^{-1} 3.1 ± 0.8	390 ± 102 g^{-1} 6.5 ± 1.7	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica plukenettii	CFR	SA	18 ± 15 g^{-1}	316 ± 81 g^{-1}	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica recta	CFR	S			BROWN and BOTHA, 1995
Erica sessiliflora	CFR	S	39.0	72.0	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Erica simulans	CFR	SA	26 ± 11 g^{-1}	54 ± 18 g^{-1}	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica sitiens	CFR	SA	140 ± 90 g^{-1} 0.9 ± 0.6	1040 ± 260 g^{-1} 6.5 ± 1.6	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica spectabilis	CFR	SA	1120 ± 80 g^{-1}	2060 ± 304 g^{-1}	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica sphaeroidea	CFR	SA	34 ± 11 g^{-1} 0.9 ± 0.3	428 ± 250 g^{-1} 11.3 ± 6.6	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica taxifolia	CFR	SA	110 ± 46 g^{-1}	180 ± 38 g^{-1}	BROWN, KOTZE and BOTHA, 1993 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica thomae	CFR	S	298 g^{-1}	1326 g^{-1}	BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica tumida	CFR	S	2 g^{-1}	20 g^{-1}	BROWN and BOTHA, 1995, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica turgida	CFR	S			BROWN and BOTHA, 1995
Erica versicolor	CFR	S	1	3	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica vestita	CFR	S	300 g^{-1}	800 g^{-1}	BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
EUPHORBIACEAE					
Phyllanthus calycinus	WA/jf WA/rm	SA/ssb SW/ssb SA/bcs	0.21 m^{-2} 2.50 $plot^{-1}$ 26.67 $plot^{-1}$	9.17 m^{-2} 7.00 $plot^{-1}$ 56.67 $plot^{-1}$	ROCHE, KOCH and DIXON, 1997
Poranthera microphylla	WA/jf	SA/ssb	0.10 m^{-2}	5.94 m^{-2}	ROCHE, KOCH and DIXON, 1997
Ricinocarpus glaucus	Aus	SA	0	20 ± 3.6	ROCHE, DIXON and PATE, 1997 BELL, 1999
FABACEAE (Papilionaceae)					
Bossiaea aquifolium	WA/rm	SW/ssb	132.17 $plot^{-1}$	150.67 $plot^{-1}$	ROCHE, KOCH and DIXON, 1997
Bossiaea ornata	WA/rm	SW PI SA	29.00 $4m^{-2}$	39.39 $4m^{-2}$ 34.94 $4m^{-2}$ 42.22 $4m^{-2}$	ROCHE, KOCH and DIXON, 1997

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Cyclopia intermedia	CFR	SW/scr	53	76	SUTCLIFFE and WHITEHEAD, 1995 BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Cyclopia longifolia	CFR	SW/ssb			DU TOIT and CAMPBELL, 1999
Gompholobium marginatum	WA/jf WA/rm	SA/ssb SW PI SA	0.62 m^{-2} 13.72 $4m^{-2}$	3.02 m^{-2} 14.22 $4m^{-2}$ 16.94 $4m^{-2}$ 19.00 $4m^{-2}$	ROCHE, KOCH and DIXON, 1997
Hovea chorizemifolia	WA/jf WA/rm	SA SA/ssb SW/ssb SA/ssb	51 ± 3.7 0.10 m^{-2} 47.00 $plot^{-1}$ 0.10 m^{-2}	66.6 ± 2.2 0.62 m^{-2} 59.33 $plot^{-1}$ 0.78 m^{-2}	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999
Hovea trisperma	Aus	SA	11.4 ± 1.3	34.6 ± 2.8	ROCHE, DIXON and PATE, 1997 BELL, 1999
Indigofera filifolia	CFR	S			BROWN and BOTHA, 2002
Kennedia coccinea	WA/rm	SW/ssb SA/ssb	9.33 $plot^{-1}$ 0.005 m^{-2}	17.33 $plot^{-1}$ 0.47 m^{-2}	ROCHE, KOCH and DIXON, 1997
Lotus uliginosus	WA/rm	SA/ssb			GRANT and KOCH, 1997
Otholobium fruticans	CFR	S	4	65	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Priestleya vestita	CFR	S			BROWN and BOTHA, 2002
Retama sphaerocarpa	MB	SA			PÉREZ-FERNÁNDEZ and RODRÍGUEZ-ECHEVERRÍA 2003
Trifolium angustifolium	MB	SA			PÉREZ-FERNÁNDEZ and RODRÍGUEZ-ECHEVERRÍA 2003
GARRYACEAE					
Garrya flavescens	SCal	CH	4	65	KEELEY, 1987
GENTIANACEAE					
Centaurium erythraea	WA/rm	SA/ssb			GRANT and KOCH, 1997
GERANIACEAE					
Geranium incanum	CFR	S	60	85	BROWN and BOTHA, 2004
Pelargonium auritum	CFR	S			BROWN and BOTHA, 1995
Pelargonium capitatum	CFR	S			BROWN and BOTHA, 1995
Pelargonium crithmifolium	CFR	S	40	80	BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Pelargonium cucullatum	CFR	S	8	15	BROWN and BOTHA, 2004
GOODENIACEAE					
Brunonia australis	Aus	SA	4.1 ± 1.2	20.2 ± 1.8	ROCHE, DIXON and PATE, 1997 BELL, 1999
Dampiera linearis	WA	SA/rm	0.67 ± 0.33 $plot^{-1}$	4 ± 1.0 $plot^{-1}$	ROKICH, DIXON, SIVASITHAMPARAM and MENEY, 2002
Goodenia geniculata	Aus	SW/ssb			ENRIGHT and KINTRUP, 2001
Lechenaultia biloba	WA WA/rm	SA SA/bcs	0 0 0.03 $plot^{-1}$	~ 40 ~ 10 0.65 $plot^{-1}$	DIXON, ROCHE and PATE, 1995 ROCHE, KOCH and DIXON, 1997 BELL, 1999

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Lechenaultia floribunda	WA	SA	0	~ 8	DIXON, ROCHE and PATE, 1995
		SA/rm	0.33 ± 0.33 plot^{-1}	2.33 ± 0.67 plot^{-1}	BELL, 1999 ROKICH, DIXON, SIVASITHAMPARAM and MENEY, 2002
Lechenaultia formosa	WA	SA	0	~ 4	DIXON, ROCHE and PATE, 1995 BELL, 1999
Lechenaultia macrantha	WA	SA	0	~ 25	DIXON, ROCHE and PATE, 1995 BELL, 1999
Scaevola calliptera	WA/rm	SA	0	~ 15	DIXON, ROCHE and PATE, 1995
		SW/ssb	0	13.8 ± 2.4	ROCHE, DIXON and PATE, 1997
			0.83 plot^{-1}	3.50 plot^{-1}	ROCHE, KOCH and DIXON, 1997 BELL, 1999
Scaevola crassifolia	Aus	SA	33.8 ± 2.4	47.3 ± 2.6	ROCHE, DIXON and PATE, 1997 BELL, 1999
Scaevola fasciculata	Aus	SA	0	3.3 ± 0.3	ROCHE, DIXON and PATE, 1997
Velleia trinervis	Aus	SA	61.3 ± 6.3	82.2 ± 2.3	ROCHE, DIXON and PATE, 1997
GYROSTEMONACEAE					
Codonocarpus cotinifolius	WA	SA	0	~ 16	DIXON, ROCHE and PATE, 1995 BELL, 1999
Gyrostemon ramulosus	WA	SA	0	~ 10	DIXON, ROCHE and PATE, 1995
Tersonia cyathiflora	Aus	SA	0	3.5 ± 0.7	ROCHE, DIXON and PATE, 1997
		SW	0	~85	BAKER, STEADMAN, PLUMMER, MERRITT and DIXON, 2005a
HYDROPHYLLACEAE					
Emmenanthe penduliflora	SCal	CH	1	49	WICKLOW, 1977
		CE	0	25	KEELEY and NITZBERG, 1984
		SA	0	20	KEELEY, MORTON, PEDROSA and TROTTER, 1985
		SS	0	100	KEELEY and PIZZORNO, 1986
			21 ± 7.6	81 ± 6.8	THANOS and RUNDEL, 1995
			8 ± 0.3	79 ± 3	KEELEY and FOTHERINGHAM, 1997, 1998a, 1998b EGERTON-WARBURTON, 1998 PRESTON, BECKER and BALDWIN, 2004
Eriodictyon crassifolium	SCal	CH	33	60	KEELEY, 1987
	SN	SW			KEELEY, McGINNIS and BOLLENS, 2005
Eucrypta chrysanthemifolia	SCal	S			KEELEY and FOTHERINGHAM, 1998b
Phacelia cicutaria	SCal	CH	17	26	KEELEY, 1984
			5	32	KEELEY, MORTON, PEDROSA and TROTTER, 1985
			9	14	
Phacelia fremontii	SCal	CH	4	11	KEELEY, MORTON, PEDROSA and TROTTER, 1985
			1	16	
Phacelia grandiflora	SCal	CH	4	8	KEELEY, MORTON, PEDROSA and TROTTER, 1985
		SA			KEELEY and FOTHERINGHAM, 1998a, 1998b
Phacelia minor	SCal	CH	0	13	KEELEY and KEELEY, 1987
		S			KEELEY and FOTHERINGHAM, 1998b
HYPERICACEAE					
Hypericum gramineum	NSW	SA/ssb	25 ± 10 m^{-2}	72 ± 23 m^{-2}	READ, BELLAIRS, MULLIGAN and LAMB, 2000
LAMIACEAE					
Ajuga australis	Aus	SW	8.3	59.2	CLARKE, DAVISON and FULLOON, 2000

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Hemigenia ramosissima	WA/jf	SA/ssb	0.00 m^{-2}	54.37 m^{-2}	ROCHE, KOCH and DIXON, 1997
Lavendula stoechas	MB/phr	CH	7	25	KEELEY and BAER-KEELEY, 1999
Prostanthera askania	Aus	SW	12	30	TIERNEY, 2006
Salvia apiana	SCal	S			KEELEY and FOTHERINGHAM, 1998b
Salvia columbariae	SCal	CH S	9	52	KEELEY, 1984 KEELEY and FOTHERINGHAM, 1998b
Salvia leucophylla	SCal	S			KEELEY and FOTHERINGHAM, 1998b
Salvia mellifera	SCal	CH S	48 18	69 69	KEELEY, 1986 KEELEY, 1987 KEELEY and FOTHERINGHAM, 1998b
LOASACEAE					
Mentzelia dispersa	SN	SW	0	~ 40	KEELEY, McGINNIS and BOLLENS, 2005
Mentzelia micrantha	SCal	S			KEELEY and FOTHERINGHAM, 1998b
MALVACEAE					
Malva neglecta		SW	28 ± 4	73 ± 12	ADKINS and PETERS, 2001 ADKINS, PETERS, PATERSON and NAVIE, 2003
MESEMBRYANTHEMACEAE					
Amphibolia hutchinsonii	CFR	S	2 ± 3	27 ± 7	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Drosanthemum lavisii	CFR	S			PIENAAR, 1995 BROWN and BOTHA, 2002
Drosanthemum midas	CFR	S			PIENAAR, 1995 BROWN and BOTHA, 2002
Drosanthemum speciosum	CFR	SW	2 ± 2.7	48 ± 15.7	PIERCE, ESLER and COWLING, 1995 BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Drosanthemum thudichumii	CFR	S	10 g_{-1}	1000 g_{-1}	PIENAAR, 1995 BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erepsia anceps	CFR	SW	1 ± 2.2	10 ± 3.5	PIERCE, ESLER and COWLING, 1995 BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erepsia lacera	CFR	SW	0.0	8 ± 10.4	PIERCE, ESLER and COWLING, 1995
Lampranthus aureus	CFR	SW	2 ± 2.7	19 ± 6.5	PIERCE, ESLER and COWLING, 1995 BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Lampranthus galpiniae	CFR	S			PIENAAR, 1995 BROWN and BOTHA, 2002
Lampranthus haworthii	CFR	SW	3 ± 2.7	10 ± 5.0	PIERCE, ESLER and COWLING, 1995 BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Lampranthus multiradiatus	CFR	SW	0.0	21 ± 9.6	PIERCE, ESLER and COWLING, 1995 BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Ruschia carolii	CFR	SW	7 ± 5.7	70 ± 7.1	PIERCE, ESLER and COWLING, 1995 BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Ruschia multiflora	CFR	SW	5 ± 7.1	23 ± 9.1	PIERCE, ESLER and COWLING, 1995 BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Ruschia promontorii	CFR	SW S	2 ± 2.7	27 ± 6.7	PIERCE, ESLER and COWLING, 1995 BROWN and BOTHA, 2002
Ruschia sarmentosa	CFR	SW	0.0	7 ± 2.7	PIERCE, ESLER and COWLING, 1995
Skiatophytum tripolium	CFR	acid,S	8	94	HICKEY and VAN JAARSVELD, 1995
MIMOSACEAE					
Acacia cyclops	Aus	SA	38.2 ± 0.7	45 ± 2.1	ROCHE, DIXON and PATE, 1997 BELL, 1999
Acacia sp.	WA/rm	SW/ssb	27.00 plot^{-1}	29.67 plot^{-1}	ROCHE, KOCH and DIXON, 1997
MOLLUGINACEAE					
Pharnaceum elongatum	CFR	S S	0	47	BROWN and BOTHA, 2002 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
MYRTACEAE					
Agonis flexuosa	Aus	S			ROCHE, DIXON and PATE, 1997 BELL, 1999
Agonis linearifolia	Aus	SA	7.9 ± 1.1	24.4 ± 1.1	ROCHE, DIXON and PATE, 1997
Astartea fascicularis	Aus	SA	6 ± 1.1	26.4 ± 1.7	ROCHE, DIXON and PATE, 1997 BELL, 1999
Baeckea camphorosmae	Aus	SA	1.7 ± 0.3	13.2 ± 0.7	ROCHE, DIXON and PATE, 1997 BELL, 1999
Calytrix breviseta	Aus	SA	7 ± 1.5	20.5 ± 1.4	ROCHE, DIXON and PATE, 1997 BELL, 1999
Calytrix depressa	Aus	SA	41.2 ± 3.8	71.3 ± 5.6	ROCHE, DIXON and PATE, 1997 BELL, 1999
Calytrix fraserii	Aus	SA	4.4 ± 0.9	12.7 ± 1.6	ROCHE, DIXON and PATE, 1997 BELL, 1999
Calytrix tetragona	Aus	SA	12.8 ± 1	25.7 ± 2.5	ROCHE, DIXON and PATE, 1997
Eucalyptus marginata	WA/jf WA/rm	SA/ssb SW/ssb	0.00 m^{-2} 2.33 plot^{-1}	1.67 m^{-2} 4.17 plot^{-1}	ROCHE, KOCH and DIXON, 1997
Hypocalymma angustifolium	WA	SA	~ 8	~ 30	DIXON, ROCHE and PATE, 1995 BELL, 1999
Kunzea ambigua	Aus	SA	~ 40	~ 60	THOMAS, MORRIS and AULD, 2003
Kunzea capitata	Aus	SA	~ 10	~ 30	THOMAS, MORRIS and AULD, 2003
Leptospermum myrsinoides	Aus	SW/ssb			ENRIGHT and KINTRUP, 2001
Leptospermum spinescens	Aus	SA	39.2 ± 4.7	88.3 ± 2.8	ROCHE, DIXON and PATE, 1997

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Scholtzia involucrata	WA	SW/fs SA/rm	2 plot^{-1} 4.33 ± 1.33 plot^{-1}	54 plot^{-1} 30 ± 10.21 plot^{-1}	LLOYD, DIXON and SIVASITHAMPARAM, 2000 ROKICH, DIXON, SIVASITHAMPARAM and MENEY, 2002
Verticordia aurea	Aus	SA	0	16.7 ± 1	ROCHE, DIXON and PATE, 1997 BELL, 1999
Verticordia chrysantha	Aus	SA	0	42.8 ± 3.8	ROCHE, DIXON and PATE, 1997 BELL, 1999
Verticordia densiflora	WA	SA	0 0	~ 65 28.1 ± 1.8	DIXON, ROCHE and PATE, 1995 ROCHE, DIXON and PATE, 1997 BELL, 1999
Verticordia eriocephala	Aus	SA	0	35.5 ± 2	ROCHE, DIXON and PATE, 1997 BELL, 1999
Verticordia huegelii	Aus	SA	0	26.2 ± 2.4	ROCHE, DIXON and PATE, 1997 BELL, 1999
Verticordia nitens	WA	SA/rm	0 plot^{-1}	11 ± 1.15 plot^{-1}	ROKICH, DIXON, SIVASITHAMPARAM and MENEY, 2002
ONAGRACEAE					
Camissonia californica	SCal	CH S	3	49	KEELEY and KEELEY, 1987 KEELEY and FOTHERINGHAM, 1998b
Clarkia epilobioides	SCal	CH	42	75	KEELEY and KEELEY, 1987
Clarkia purpurea	SCal	CH	40	72	KEELEY and KEELEY, 1987
OXALIDACEAE					
Oxalis comiculata	WA/rm	SA/ssb			GRANT and KOCH, 1997
PAPAVERACEAE					
Papaver californicum	SCal	CH	0	89	KEELEY and KEELEY, 1987
Romneya coulteri	SCal	CH S	0	40	KEELEY and KEELEY, 1987 KEELEY and FOTHERINGHAM, 1998b
Romneya trichocalyx	SCal	CH	0 0	24 17	KEELEY, 1987
PENAEACEAE					
Endonema retzioides	CFR	S	36	86	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Penaea sp.	CFR	S S	4 ± 3	28 ± 8	BROWN and BOTHA, 2002 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
PITTOSPORACEAE					
Billardiera bicolor	WA	SA	~ 8	~ 50	DIXON, ROCHE and PATE, 1995 BELL, 1999
Billardiera coeruleo-punctata	WA/rm	SW PI SA	7.89 4m^{-2}	17.28 4m^{-2} 36.61 4m^{-2} 46.83 4m^{-2}	ROCHE, KOCH and DIXON, 1997
Billardiera variifolia	Aus WA/rm	SA, storage SA/bcs	0 0.25 plot^{-1}	26.7 ± 2.3 3.00 plot^{-1}	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999
Cheiranthera preissiana	Aus	SA	61.8 ± 3.3	91.8 ± 2.6	ROCHE, DIXON and PATE, 1997 BELL, 1999
Sollya heterophylla	Aus	SA	36.1 ± 2.5	70.8 ± 4.5	ROCHE, DIXON and PATE, 1997 BELL, 1999

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
POLEMONIACEAE					
Allophyllum glutinosum	SCal	S			KEELEY and FOTHERINGHAM, 1998b
Gilia australis	SCal	CH	31	80	KEELEY and KEELEY, 1987
Gilia capitata	SCal	CH	8 20	83 69	KEELEY and KEELEY, 1987
POLYGALACEAE					
Comesperma virgatum	Aus	SA	11.7 ± 2	30.7 ± 4.3	ROCHE, DIXON and PATE, 1997 BELL, 1999
Nylandtia spinosa	CFR	S			BROWN and BOTHA, 2002
POLYGONACEAE					
Fallopia convolvulus		SW	15 ± 1	24 ± 2	ADKINS and PETERS, 2001 ADKINS, PETERS, PATERSON and NAVIE, 2003
Polygonum pennsylvanicum		SW	9 ± 1	21 ± 1	ADKINS and PETERS, 2001 ADKINS, PETERS, PATERSON and NAVIE, 2003
Polygonum persicaria		SW	21 ± 2	39 ± 2	ADKINS and PETERS, 2001 ADKINS, PETERS, PATERSON and NAVIE, 2003
PRIMULACEAE					
Anagallis arvensis	NSW	SA/ssb	4 ± 3 m^{-2}	77 ± 36 m^{-2}	READ, BELLAIRS, MULLIGAN and LAMB, 2000
PROTEACEAE					
Aulax cancellata	CFR	S			BROWN and BOTHA, 1995
Banksia attenuata	WA	SA/ssb	0 $plot^{-1}$	1 ± 0.58 $plot^{-1}$	ROKICH, DIXON, SIVASITHAMPARAM and MENEY, 2002
Banksia grandis	WA/jf WA/rm	SA/ssb SA/bcs	0.10 m^{-2} 11.83 $plot^{-1}$	0.73 m^{-2} 26.5 $plot^{-1}$	ROCHE, KOCH and DIXON, 1997
Conospermum incurvum	WA	SA	0 0	3 5.3 ± 0.8	DIXON, ROCHE and PATE, 1995 ROCHE, DIXON and PATE, 1997 BELL, 1999
Conospermum stoechadis	WA	SA/ssb	0.67 ± 0.33 $plot^{-1}$	4.67 ± 3.71 $plot^{-1}$	ROKICH, DIXON, SIVASITHAMPARAM and MENEY, 2002
Conospermum triplinervium	WA	SA	0 0	~ 8 2.9 ± 0.6	DIXON, ROCHE and PATE, 1995 ROCHE, DIXON and PATE, 1997
Grevillea buxifolia	NSW	SA	0 0	~ 25 ~ 40	KENNY, 2000 MORRIS, 2000
Grevillea diffusa	NSW	SA	0	~ 45	MORRIS, 2000
Grevillea juniperina	NSW	SA	~ 5	~ 30	MORRIS, 2000
Grevillea linearifolia	NSW	SA SW	~ 13 ~ 20	~ 55 ~ 50	MORRIS, 2000 MORRIS, TIEU and DIXON, 2000
Grevillea mucronulata	NSW	SA	~ 20	~ 60	MORRIS, 2000
Grevillea polybotrya	Aus	SA	7.2 ± 1.3	18.8 ± 1.7	ROCHE, DIXON and PATE, 1997 BELL, 1999
Grevillea quercifolia	Aus	SA	1.6 ± 1	19.8 ± 1.5	ROCHE, DIXON and PATE, 1997 BELL, 1999
Grevillea sericea	NSW	SA	~ 20 ~10	~ 60 ~ 45	KENNY, 2000 MORRIS, 2000

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Grevillea wilsonii	WA/rm	SA/bcs SW PI	~ 10 4.5 ± 1.6 - 0.88 $4m^{-2}$	~ 80 34.8 ± 5.1 2.75 $plot^{-1}$ 1.44 $4m^{-2}$ 5.33 $4m^{-2}$ 8.67 $4m^{-2}$	DIXON, ROCHE and PATE, 1995 ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999
Grevillia speciosa	NSW	SA	~ 50 ~ 20	~ 90 ~ 40	KENNY, 2000 MORRIS, 2000
Hakea corymbosa	Aus	SA	19.5 ± 3	75.7 ± 6	ROCHE, DIXON and PATE, 1997 BELL, 1999
Hakea undulata	WA/rm	SA/bcs SW PI SA	20.00 $plot^{-1}$ 85.50 $4m^{-2}$	23.67 $plot^{-1}$ 81.80 $4m^{-2}$ 85.5 $4m^{-2}$ 101.8 $4m^{-2}$	ROCHE, KOCH and DIXON, 1997
Isopogon ceratophyllous	Aus	SA	0	22.2 ± 5.1	ROCHE, DIXON and PATE, 1997 BELL, 1999
Leucadendron arcuatum	CFR	S	3	49	BROWN and BOTHA, 2004
Leucadendron conicum	CFR	S	59	80	BROWN and BOTHA, 2004
Leucadendron coniferum	CFR	S	79	98	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Leucadendron daphnoides	CFR	S/scr	10	53	BROWN and BOTHA, 2004
Leucadendron rubrum	CFR	S	20	61	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Leucadendron salignum	CFR	S	47	75	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Leucadendron tinctum	CFR	S	21	83	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Leucospermum prostratum	CFR	S	4	21	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Lysinema ciliatum	WA	SA SW	0 0	~ 45 ~ 90	DIXON, ROCHE and PATE, 1995 BELL, 1999
Petrophile drummondii	WA	SA	~ 25 22.5 ± 2.2	~ 90 62.5 ± 5.2	DIXON, ROCHE and PATE, 1995 ROCHE, DIXON and PATE, 1997 BELL, 1999
Protea acuminata	CFR	S			BROWN and BOTHA, 2002
Protea compacta	CFR	SW	68 ± 5 65	88 ± 4 96	BROWN, 1993a BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Protea cordata	CFR	S	68	74	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Serruria florida	CFR	S			BROWN and BOTHA, 1995
Serruria phylicoides	CFR	SW S	3 ± 1	27 ± 9	BROWN, 1993a BROWN and BOTHA, 1995 BROWN and BOTHA, 2002 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Serruria villosa	CFR	S	1	8	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Stirlingia latifolia	WA	SA	0 0 0.6 ± 0.3 plot^{-1}	~ 45 6.5 ± 0.5 13 ± 2 plot^{-1}	DIXON, ROCHE and PATE, 1995 ROCHE, DIXON and PATE, 1997 ROCHE, DIXON and PATE, 1998 BELL, 1999
RANUNCULACEAE					
Clematis glycinoides	QL	SA	5 sample^{-1}	21 sample^{-1}	TANG, BOULTER and KITCHING, 2003
Clematis lasiantha	SCal	CH	32 0	79 16	KEELEY, 1987
Clematis pubescens	WA/rm	SA SA/bcs	0 12.75 plot^{-1}	22.7 ± 1.7 25.25 plot^{-1}	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999
Clematis vitalba	MB	SA			CROSTI, LADD, DIXON and PIOTTO, 2006
RHAMNACEAE					
Rhamnus alaternus	MB	SA			CROSTI, LADD, DIXON and PIOTTO, 2006
Rhamnus crocea	SCal	CH	3	32	KEELEY, 1987
Siegfriedia darwinioides	WA	SA	< 4	< 8	DIXON, ROCHE and PATE, 1995 BELL, 1999
Spyridium globosum	WA	SA	~ 8	~ 20	DIXON, ROCHE and PATE, 1995 BELL, 1999
Spyridium parvifolium	Aus	SA	0.2 ± 0.1	1.2 ± 0.2	ROCHE, DIXON and PATE, 1997
Trymalium ledifolium	WA/rm WA/jf	SW/ssb SA/ssb	8.17 plot^{-1} 0.05 m^{-2}	16.17 plot^{-1} 0.52 m^{-2}	ROCHE, KOCH and DIXON, 1997 WARD, KOCH and GRANT, 1997
ROSACEAE					
Adenostoma fasciculatum	SCal SN	CH SW	4	11	KEELEY, 1987 KEELEY, McGINNIS and BOLLENS, 2005
Cliffortia ruscifolia	CFR	S S	0	28 ± 2	BROWN and BOTHA, 2002 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Purshia tridentata	US	SA			BLANK and YOUNG, 1998
RUBIACEAE					
Galium angustifolium	SCal	CH	22	43	KEELEY and KEELEY, 1987
Opercularia diphylla	NSW	SA/ssb	9 ± 4 m^{-2}	31 ± 10 m^{-2}	READ, BELLAIRS, MULLIGAN and LAMB, 2000
Opercularia echinocephala	WA/jf WA/rm	SA/ssb SW/ssb SA/bcs	0.00 m^{-2} 24.67 plot^{-1} 12.50 plot^{-1}	1.56 m^{-2} 40.83 plot^{-1} 27.50 plot^{-1}	GRANT and KOCH, 1997 ROCHE, KOCH and DIXON, 1997
Opercularia sp.	Aus	SW/ssb			ENRIGHT and KINTRUP, 2001
Opercularia vaginata	WA	SA/ssb	0 plot^{-1}	6 ± 3.79 plot^{-1}	ROKICH, DIXON, SIVASITHAMPARAM and MENEY, 2002
RUTACEAE					
Agathosma ovata	CFR	S	4	10	BROWN and BOTHA, 2002, 2004
Boronia fastigiata	WA/rm	SW/ssb	17.83 plot^{-1}	27.67 plot^{-1}	ROCHE, KOCH and DIXON, 1997
Boronia ramosa	WA	SA/ssb	0 plot^{-1}	1.33 ± 0.33 plot^{-1}	ROKICH, DIXON, SIVASITHAMPARAM and MENEY, 2002
Boronia safrolifera		SW			ADKINS, PETERS, PATERSON and NAVIE, 2003
Boronia tenuis	Aus	SA	3.2 ± 3.2	57.1 ± 16	ROCHE, DIXON and PATE, 1997 BELL, 1999

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Boronia viminea	Aus	SA	1.1 ± 0.2	4.4 ± 1.2	ROCHE, DIXON and PATE, 1997 BELL, 1999
Diplolaena dampieri	Aus	SA	8.1 ± 0.8	44.2 ± 3.8	ROCHE, DIXON and PATE, 1997 BELL, 1999
Eristemon spicatus	WA	SA	0	~ 5	DIXON, ROCHE and PATE, 1995 BELL, 1999
Geleznowia verrucosa	WA	SA SW	0 0 0.4 ± 0.4	~ 20 ~ 70 43.5 ± 2.6	DIXON, ROCHE and PATE, 1995 ROCHE, DIXON and PATE, 1997 BELL, 1999
Zieria laxiflora					ADKINS, PETERS, PATERSON and NAVIE, 2003
SANTALACEAE					
Exocarpos sparteus	Aus	SA	0	13.3 ± 1.4	ROCHE, DIXON and PATE, 1997 BELL, 1999
Leptomeria cunninghamii	Aus	SA	0	1.7 ± 0.3	ROCHE, DIXON and PATE, 1997 BELL, 1999
SCROPHULARIACEAE					
Antirrhinum coulterianum	SCal	CH CH S	0 2	12 42	KEELEY, MORTON, PEDROSA and TROTTER, 1985 KEELEY and KEELEY, 1987 KEELEY and FOTHERINGHAM, 1998b
Antirrhinum kelloggii	SCal	CH S	39	63	KEELEY and KEELEY, 1987 KEELEY and FOTHERINGHAM, 1998b
Antirrhinum nuttallianum	SCal	S			KEELEY and FOTHERINGHAM, 1998b
Chenopodiopsis chenopodiodes	CFR	S			BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Chenopodiopsis hirta	CFR	S			BROWN and BOTHA, 2002, 2004
Collinsia parryi	SCal	CH	24	77	KEELEY and KEELEY, 1987
Dischisma capitatum	CFR	S	8 ± 4	20 ± 2	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Hebenstreitia paarlensis	CFR	S			BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Manulea cheiranthus	CFR	S	2 ± 1	32 ± 4	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Mimulus bolanderi	SN	SW	~ 10	~ 40	KEELEY, McGINNIS and BOLLENS, 2005
Mimulus clevelandii	SCal	S			KEELEY and FOTHERINGHAM, 1998b
Nemesia lucida	CFR	S	12 ± 4	24 ± 6	BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Nemesia versicolor	CFR	S			BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Penstemon centranthifolius	SCal	CH S	0	16	KEELEY and KEELEY, 1987 KEELEY and FOTHERINGHAM, 1998b
Penstemon heterophyllus	SCal	CH	54	74	KEELEY and KEELEY, 1987
Penstemon spectabilis	SCal	CH	1	61	KEELEY and KEELEY, 1987

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Selago sp.	CFR	S			BROWN and BOTHA, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Veronica persica		SW	79 ± 3	100 ± 0	ADKINS and PETERS, 2001 ADKINS, PETERS, PATERSON and NAVIE, 2003
Zaluzianskya sp.	CFR	S			BROWN and BOTHA, 2002
Zaluzianskya villosa	CFR	S	1	23	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
SOLANACEAE					
Nicotiana attenuata	US	SA SW SW/cls SW/ssb	0	73	BALDWIN, STASZAK-KOZINSKI and DAVIDSON, 1994 BALDWIN and MORSE, 1994 PRESTON and BALDWIN, 1999 PRESTON, BECKER and BALDWIN, 2004 SCHWACHTJE and BALDWIN, 2004
STACKHOUSIACEAE					
Stackhousia pubescens	WA	SA	< 4 20.6 ± 1.8	~ 8 47.7 ± 4	DIXON, ROCHE and PATE, 1995 ROCHE, DIXON and PATE, 1997 BELL, 1999
Tripterococcus brunonis	WA/jf WA/rm	SA SA/ssb SW/ssb	0 0.00 m^{-2} -	3.8 ± 0.9 3.65 m^{-2} 2.83 $plot^{-1}$	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999
STERCULIACEAE					
Lasiopetalum floribundum	Aus WA/jf	SA SA/ssb	0.7 ± 0.3 -	2.9 ± 0.5 -	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997
Rulingia platycalyx	WA	SA	~ 8	~ 20	DIXON, ROCHE and PATE, 1995 BELL, 1999
STILBACEAE					
Stilbe vestita	CFR	S S	1	3	BROWN and BOTHA, 2002 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
STYLIDACEAE					
Stylidium soboliferum	Aus	SW/ssb			ENRIGHT and KINTRUP, 2001
Stylidium sp.	WA	SA/ssb SA/rm	0.33 ± 0.33 $plot^{-1}$ 4.67 ± 1.86 $plot^{-1}$	21.3 ± 13.8 $plot^{-1}$ 41 ± 5.13 $plot^{-1}$	ROKICH, DIXON, SIVASITHAMPARAM and MENEY, 2002
Levenhookia pusilla	WA/jf WA/rm	SA/ssb	0.10 m^{-2}	30.31 m^{-2}	GRANT and KOCH, 1997 ROCHE, KOCH and DIXON, 1997
Stylidium affine	Aus WA	SA SW PI	0 5.3 ± 0.8	~ 70 68.6 ± 2.9	ROCHE, DIXON and PATE, 1997 BELL, 1999 TIEU, DIXON, SIVASITHAMPARAM, PLUMMER and SIELER, 1999 TIEU, DIXON, MENEY and SIVASITHAMPARAM, 2001a, 2001b
Stylidium amoenum	Aus	SA	7.4 ± 1.4	28.8 ± 2.2	ROCHE, DIXON and PATE, 1997 BELL, 1999
Stylidium brunonianum	Aus WA	SA SW PI	< 5 4 ± 1.5 $plot^{-1}$ 13.8 ± 1.3	~ 40 13 ± 2 $plot^{-1}$ 44.6 ± 5.8	ROCHE, DIXON and PATE, 1997 ROCHE, DIXON and PATE, 1998 BELL, 1999 TIEU, DIXON, SIVASITHAMPARAM, PLUMMER and SIELER, 1999

Species	Habitat	Treatment	Control germination	Treatment germination	Reference
Stylidium bulbiferum	Aus	SA	3.9 ± 0.9	15.9 ± 0.7	ROCHE, DIXON and PATE, 1997 BELL, 1999
Stylidium calcaratum	WA/jf	SA/ssb	0.62 m^{-2}	17.08 m^{-2}	GRANT and KOCH, 1997 ROCHE, KOCH and DIXON, 1997
Stylidium crossocephalum	WA	SA	0	~ 30	TIEU, DIXON, MENEY and SIVASITHAMPARAM, 2001b
Stylidium hispidum	WA	SA	8.8 ± 1 1 ± 0.5 $plot^{-1}$	33 ± 1.8 25 ± 3 $plot^{-1}$	ROCHE, DIXON and PATE, 1997 ROCHE, DIXON and PATE, 1998 BELL, 1999
Stylidium junceum	WA/rm	SW PI SA	11.2 ± 1.5 7.28 $4m^{-2}$	82 ± 3.7 38.44 $4m^{-2}$ 54.38 $4m^{-2}$ 86.67 $4m^{-2}$	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999
Stylidium repens	WA	SA	3 ± 0.8 $plot^{-1}$	38 ± 4 $plot^{-1}$	ROCHE, DIXON and PATE, 1998 BELL, 1999
Stylidium schoenoides	WA/jf	SA/ssb SA	0.00 m^{-2} 3.8 ± 0.7 0.0 $plot^{-1}$	0.42 m^{-2} 55.2 ± 2.6 9 ± 1.7 $plot^{-1}$	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 ROCHE, DIXON and PATE, 1998 BELL, 1999
THYMELAEACEAE					
Gnidia pinifolia	CFR	S	2	5	BROWN and BOTHA, 2002, 2004
Passerina vulgaris	CFR	S			BROWN and BOTHA, 1995, 2002, 2004 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Pimelea ciliata	Aus	SA	0	10.3 ± 0.9	ROCHE, DIXON and PATE, 1997 BELL, 1999
Pimelea imbricata	Aus	SA	1.1 ± 0.7	14 ± 1.5	ROCHE, DIXON and PATE, 1997 BELL, 1999
Pimelea leucantha	Aus	SA	0.7 ± 0.5	3.2 ± 0.6	ROCHE, DIXON and PATE, 1997 BELL, 1999
Pimelea spectabilis	WA	SA	0	~ 25	DIXON, ROCHE and PATE, 1995 BELL, 1999
Pimelea suaveolens	Aus WA/rm	SA SW/ssb SA/bcs	0 - -	23.3 ± 3.3 1.17 $plot^{-1}$ 1.00 $plot^{-1}$	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999
Pimelea sylvestris	WA	SA	0	~ 15 ~ 35	DIXON, ROCHE and PATE, 1995 BELL, 1999
Struthiola myrsinites	CFR	S	10	33	BROWN and BOTHA, 2002, 2004
TREMANDRACEAE					
Tetratheca hirsuta	WA/rm	SA SW/ssb SA/bcs	~ 20 13.2 ± 0.8 2.67 $plot^{-1}$ 5.50 $plot^{-1}$	~ 30 30 ± 1.7 6.83 $plot^{-1}$ 11.50 $plot^{-1}$	DIXON, ROCHE and PATE, 1995 ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999
Tetratheca juncea	WA	SW	20 ± 5	49 ± 5	BELLAIRS, BARTIER, GRAVINA and BAKER, 2006
VERBENACEAE					
Verbena officinalis	QL	SA/ssb			ADKINS, DAVIDSON, MATTHEW, NAVIE, WILLS, TAYLOR and BELLAIRS, 2000
VIOLACEAE					
Hybanthus calycinus	Aus	SA	0	6.6 ± 0.7	ROCHE, DIXON and PATE, 1997 BELL, 1999
Hybanthus floribundus	WA	SA	0	~ 4	DIXON, ROCHE and PATE, 1995 BELL, 1999

APPENDIX 2
List of species which do not show a significant germination response to smoke treatments

Species in which seed germination was NOT significantly stimulated by smoke, smoke solutions, charred wood or aqueous extracts of charred wood.

Habitat

Aus = Australia
CFR = Cape Floristic Region, South Africa
jf = jarrah forest
MB = Mediterranean Basin
rm = rehabilitated mine soils
phr = phrygana community
SA = South Africa
SCal = southern California
SN = Sierra Nevada
WA = western Australia

Treatment

bcs = broadcast seed (rehabilitated mine soils)
CE = charate extract (extract from powdered charred wood)
CH = charate (powdered charred wood)
SA = aerosol smoke
SC = smoke-derived germination compound, 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one
SS = smoke-treated substrate (e.g. sand, filter paper)
ssb = soil seed bank
SW = smoke water

Notes

No germination data have been given in this appendix. Please see the related reference for detailed information. It should be noted, however, that this list includes species that:

- generally gave a low or nil germination percentage, and smoke treatments did not improve germination
- showed a reduction in germination following smoke treatments
- germinated without any treatment, provided the temperature was favourable, and smoke treatments did not improve germination any further

Species	Habitat	Treatment	Reference
Gymnospermae			
CUPRESSACEAE			
Juniperus oxycedrus macrocarpa	MB	SA	CROSTI, LADD, DIXON and PIOTTO, 2006
Widdringtonia cupressoides (syn. *Widdringtonia nodiflora*)	CFR	SA S	BROWN, 1993a BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Monocotyledonae			
AGAPANTHACEAE			
Agapanthus africanus	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
ALLIACEAE			
Allium praecox	SCal	CH	KEELEY and KEELEY, 1987
Allium sp.	MB/phr	CH	KEELEY and BAER-KEELEY, 1999
AMARYLLIDACEAE			
Bloomeria crocea	SCal	CH	KEELEY and KEELEY, 1987
Cyrtanthus ventricosus	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Dichelostemma pulchella	SCal	CH	KEELEY and KEELEY, 1987
ANTHERICACEAE			
Agrostocrinum scabrum	WA/jf	CH SA	BELL, VLAHOS and WATSON, 1987 ROCHE, DIXON and PATE, 1997
Caesia calliantha	Aus	SA	ROCHE, DIXON and PATE, 1997
Caesia parviflora	WA/rm	SA/bcs	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997
Chamaescilla corymbosa	WA/rm	SW/ssb SA/bcs	ROCHE, KOCH and DIXON, 1997
Johnsonia lupulina	Aus	SA	ROCHE, DIXON and PATE, 1997
Sowerbaea multicaulis	Aus	SA	ROCHE, DIXON and PATE, 1997
Thysanotus dichotomus	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Thysanotus fastigiatus	WA/rm	SW/ssb SA/bcs	ROCHE, KOCH and DIXON, 1997
Thysanotus multiflorus	WA/jf	CH SA/ssb	BELL, VLAHOS and WATSON, 1987 WARD, KOCH and GRANT, 1997
Thysanotus thyrsoides	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
Tricoryne elatior	WA/rm	SA/bcs	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999
ASPHODELACEAE			
Asphodelus aestivus	MB/phr	CH	KEELEY and BAER-KEELEY, 1999
Asphodelus ramosus	MB	SA	CROSTI, LADD, DIXON and PIOTTO, 2006
Bulbine caulescens	-	SW/CH	BRINGMANN, NOLL and RISCHER, 2002
Kniphofia uvaria	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Trachyandra sp.	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
COLCHICACEAE			
Burchardia umbellata	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
CYPERACEAE			
Cyathochaeta avenacea	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
Cyperus gracilis	NSW	SA/ssb	READ, BELLAIRS, MULLIGAN and LAMB, 2000
Gahnia clarkei	Aus	SA	ROCHE, DIXON and PATE, 1997

Species	Habitat	Treatment	Reference
Isolepis incomptula	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Lepidosperma angustatum	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Lepidosperma longitudinale	Aus	SA	ROCHE, DIXON and PATE, 1997
Lepidosperma tenue	WA/jf	SA/ssb	WARD, KOCH and GRANT, 1997
Mariscus thunbergii	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Tetraria capillaris	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
Tetraria octandra	Aus	SA	ROCHE, DIXON and PATE, 1997
DASYPOGONACEAE			
Acanthocarpus preissii	Aus	SA	ROCHE, DIXON and PATE, 1997
Dasypogon bromeliifolius	Aus	SA	ROCHE, DIXON and PATE, 1997
Kingia australis	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Lomandra integra	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Lomandra micrantha	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Lomandra nigricans	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Lomandra preissii	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Lomandra purpurea	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Lomandra sericea	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
Lomandra sonderi	WA/rm	SA SW/ssb	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999
Lomandra sp.	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Lomandra spartea	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
ECDEIOCOLEACEAE			
Ecdeiocolea monostachya	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
HAEMODORACEAE			
Anigozanthos manglesii	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Conostylis aculeata	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Conostylis setosa	WA/jf WA.rm	CH SA/bcs	BELL, VLAHOS and WATSON, 1987 ROCHE, KOCH and DIXON, 1997
Dilatris pillansii	CFR	S	BROWN and BOTHA, 2004
Haemodorum laxum	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Macropidia fuliginosa	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Wachendorfia paniculata	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
HYACINTHACEAE			
Albuca flaccida	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Albuca sp.	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
IRIDACEAE			
Aristea major	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Bobartia gladiata	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004

Species	Habitat	Treatment	Reference
Bobartia gladiata subsp. *gladiata*	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Geissorhiza sp.	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Moraea ochroleuca	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Moraea ramosissima	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Nivenia stokoei	CFR	S	BROWN and BOTHA, 2004
Orthosanthus laxus	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Patersonia babianoides	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Patersonia juncea	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Patersonia occidentalis	WA/rm	SW/ssb SA/bcs	ROCHE, KOCH and DIXON, 1997
Patersonia pygmaea	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
Patersonia rudis	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Patersonia sericea	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Patersonia umbrosa	WA/rm	SA/bcs	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997
Pillansia templemannii	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Romulea sp.	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Tritioniopsis parviflora	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Tritioniopsis triticea	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Watsonia borbonica	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Watsonia tabularis	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
LANARIACEAE			
Lanaria lanata	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
LILIACEAE			
Calochortus concolor	SCal	CH	KEELEY and KEELEY, 1987
Calochortus splendens	SCal	CH	KEELEY and KEELEY, 1987
Chlorogalum parviflorum	SCal	CH	KEELEY and KEELEY, 1987
Zigadenus fremontii	SCal	CH	KEELEY, MORTON, PEDROSA and TROTTER, 1985
ORCHIDACEAE			
Pterostylis sp.	WA/jf	SA/ssb	WARD, KOCH and GRANT, 1997
PHORMIACEAE			
Dianella amoena	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Dianella callicarpa	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Dianella revoluta	WA/jf WA/rm	CH SW/ssb SA/bcs	BELL, VLAHOS and WATSON, 1987 ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999
Dianella tasmanica	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Stypandra glauca	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999

Species	Habitat	Treatment	Reference
POACEAE			
Aira elegans	Aus	SW/ssb	ENRIGHT and KINTRUP, 2001
Aristida ramosa	Aus	SW	CLARKE and FRENCH, 2005
Aristida vagans	Aus	SW	CLARKE and FRENCH, 2005
Austrodanthonia racemosa var. *racemosa*	Aus	SW	CLARKE and FRENCH, 2005
Austrostipa rudis ssp. *rudis setacea*	Aus	SW	CLARKE and FRENCH, 2005
Avena barbata	SCal	CH	KEELEY, MORTON, PEDROSA and TROTTER, 1985
Bothriochloa decipiens	NSW	SW	READ and BELLAIRS, 1999
Bothriochloa macra	Aus	SW	CLARKE and FRENCH, 2005
Bromus diandrus		SW	ADKINS, PETERS, PATERSON and NAVIE, 2003
Chloris truncata	NSW	SW	READ and BELLAIRS, 1999
Cymbopogon refractus	NSW	SW	READ and BELLAIRS, 1999 CLARKE and FRENCH, 2005
Danthonia caespitosa	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Danthonia eriantha	NSW	SW	READ and BELLAIRS, 1999
Danthonia linkii var. *fulva*	NSW	SW	READ and BELLAIRS, 1999
Danthonia pallida	NSW	SW	READ and BELLAIRS, 1999
Danthonia racemosa	NSW	SW	READ and BELLAIRS, 1999
Dichanthium sericeum	Aus	SW	CLARKE and FRENCH, 2005
Dichelachne micrantha	Aus	SW	CLARKE and FRENCH, 2005
Digitaria brownii	NSW	SW	READ and BELLAIRS, 1999
Digitaria ramularis	Aus	SW	CLARKE and FRENCH, 2005
Echinopogon caespitosus var. *caespitosus*	Aus	SW	CLARKE and FRENCH, 2005
Ehrharta calycina	Aus (SA)	SW	SMITH, BELL and LONERAGAN, 1999
Entolasia stricta	Aus	SW	CLARKE and FRENCH, 2005
Eragrostis elongata	NSW	SW	READ and BELLAIRS, 1999
Eriochloa pseudoacrotricha	NSW	SW	READ and BELLAIRS, 1999
Melica imperfecta	SCal	CH	KEELEY and KEELEY, 1987
Microlaena stipoides	NSW	SW	READ and BELLAIRS, 1999
Microlaena stipoides var. *stipoides*	Aus	SW	CLARKE and FRENCH, 2005
Neurachne alopecuroidea	WA/jf	SA/ssb	WARD, KOCH and GRANT, 1997
Paspalidium distans	Aus	SW	CLARKE and FRENCH, 2005
Sporobolus indicus var. *capensis*	Aus	SW	CLARKE and FRENCH, 2005
Stipa coronata	SCal	CH	KEELEY, MORTON, PEDROSA and TROTTER, 1985
Stipa lepida	SCal	CH	KEELEY and KEELEY, 1987
Stipa scabra subsp. *scabra*	NSW	SW	READ and BELLAIRS, 1999
Tetrarrhena laevis	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
Themeda australis	Aus	SW	CLARKE and FRENCH, 2005
RESTIONACEAE			
Askidiosperma chartaceum	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Askidiosperma esterhuyseniae	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Calopsis impolita	CFR	SA S	BROWN, 1993a BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Calopsis paniculata	CFR	SA S	BROWN, JAMIESON and BOTHA, 1994 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004

Species	Habitat	Treatment	Reference
Cannomois parviflora	CFR	SA S	BROWN, JAMIESON and BOTHA, 1994 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Cannomois taylorii	CFR	S	BROWN and BOTHA, 2004
Ceratocaryum argenteum	CFR	S	BROWN and BOTHA, 2004
Chondropetalum tectorum	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Elegia caespitosa	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Elegia capensis	CFR	SA S	BROWN, JAMIESON and BOTHA, 1994 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Elegia grandispicata	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Elegia stipularis	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Elegia thyrsifera	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Hydrophyilus rattrayi	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Hypodiscus neesii	CFR	SA S	BROWN, JAMIESON and BOTHA, 1994 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Hypodiscus striatus	CFR	SA S	BROWN, JAMIESON and BOTHA, 1994 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Ischyrolepis subverticillata	CFR	SA	McCARTAN, RAMAROSANDRATANA and VAN STADEN, 2004
Mastersiella digitata	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Restio brachiatus	CFR	SW SA	BROWN, 1993a BROWN, JAMIESON and BOTHA, 1994 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Restio pachystachyus	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Restio praeacutus	CFR	SA S	BROWN, 1993a BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Restio similis	CFR	SA	BROWN, 1993a
Rhodocoma gigantea	CFR	SW	BROWN, 1993a
Thamnochortus erectus	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Thamnochortus lucens	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Thamnochortus platypteris	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Thamnochortus punctatus	CFR	SA	BROWN, 1993a
Thamnochortus rigidus	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Willdenowia incurvata	CFR	SW SA	BR\OWN, 1993a BROWN, JAMIESON and BOTHA, 1994 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
XANTHORRHOEACEAE			
Xanthorrhoea gracilis	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Xanthorrhoea preissii	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Xanthorrhoea sp.	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997

Species	Habitat	Treatment	Reference
Dicotyledonae			
AMARANTHACEAE			
Ptilotus auriculifloius	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Ptilotus axillaris	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Ptilotus drummondii	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Ptilotus exaltatus	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Ptilotus helipterioides	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Ptilotus nobilis	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Ptilotus polystachys	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Ptilotus rotindifolius	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
ANACARDIACEAE			
Malosoma laurina	SCal	CH	KEELEY, 1987
Rhus tomentosa	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
APIACEAE			
Actinotus leucocephalus	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Ammoides pusilla	MB/phr	CH	KEELEY and BAER-KEELEY, 1999
Angelica sylvestris		SW	ADKINS and PETERS, 2001 ADKINS, PETERS, PATERSON and NAVIE, 200
Apiastrum angustifolium	SCal	CH	KEELEY, MORTON, PEDROSA and TROTTER, 1985
Daucus carota	MB/phr	CH	KEELEY and BAER-KEELEY, 1999
Daucus glochidiatus	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Daucus pusillus	SCal	CH	KEELEY and KEELEY, 1987
Heracleum sphondylium		SW	ADKINS and PETERS, 2001 ADKINS, PETERS, PATERSON and NAVIE, 2003
Hydrocotyle callicarpa	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
Lomatium dasycarpum	SCal	CH	KEELEY and KEELEY, 1987
Pentapeltis peltigera	WA/rm WA/jf	SA SW/ssb SA/ssb	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 WARD, KOCH and GRANT, 1997 BELL, 1999
Platysace compressa	WA/rm WA/jf	SW/ssb SA/bcs SA/ssb	ROCHE, KOCH and DIXON, 1997 WARD, KOCH and GRANT, 1997
Platysace tenuissima	WA/rm WA/jf	SW/ssb SA/bcs SA/ssb	ROCHE, KOCH and DIXON, 1997 WARD, KOCH and GRANT, 1997
Trachymene pilosa	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Xanthosia atkinsoniana	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Xanthosia candida	WA/rm WA/jf	SW/ssb SA/ssb	ROCHE, KOCH and DIXON, 1997 WARD, KOCH and GRANT, 1997
Xanthosia huegelii	WA/jf	SA/ssb	WARD, KOCH and GRANT, 1997

Species	Habitat	Treatment	Reference
APOCYNACEAE			
Alyxia buxifolia	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
ASTERACEAE			
Arctotis acaulis	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Atractylis cancellata	MB/phr	CH	KEELEY and BAER-KEELEY, 1999
Brachycome muelleri	Aus	SW	JUSAITIS, POLOMKA and SORENSEN, 2004
Chrysocoma coma-aurea	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Conyza albida	WA/jf	SA/ssb	WARD, KOCH and GRANT, 1997
Cotula turbinata	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Dimorphotheca nudicaulis (syn. *Castalis nudicaulis*)	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Disparago ericoides	CFR	S	BROWN and BOTHA, 2004
Edmondia fasciculata	CFR	S	BROWN and BOTHA, 2004
Erymophyllum slossanthus	WA	SC SW	MERRITT, KRISTIANSEN, FLEMATTI, TURNER, GHISALBERTI, TRENGOVE and DIXON, 2006
Euryops abrotanifolius	CFR	S	BROWN and BOTHA, 2004
Euryops speciosissimus	CFR	S	BROWN and BOTHA, 2004
Euryops virgineus	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Felicia aethiopica subsp. *aethiopica*	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Felicia echinata	Fynbos	CH	PIERCE and MOLL, 1994
Felicia filifolia	CFR	S	BROWN and BOTHA, 2004
Gnephosis acicularis	WA	SC SW	MERRITT, KRISTIANSEN, FLEMATTI, TURNER, GHISALBERTI, TRENGOVE and DIXON, 2006
Haplopappus squarrosus	SCal	CH	KEELEY, 1987
Hedypnois cretica	MB/phr	CH	KEELEY and BAER-KEELEY, 1999
Helichrysum aureonitens	SA	SS	AFOLAYAN, MEYER and LEEUWNER, 1997
Helichrysum dasyanthum	CFR	S	BROWN and BOTHA, 2004
Helichrysum pandurifolium	CFR	S	BROWN and BOTHA, 2004
Helichrysum patulum	CFR	S	BROWN and BOTHA, 2004
Heterotheca grandiflora	SCal	CH	KEELEY and KEELEY, 1987
Hirpicium alienatum	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Hymenolepis parviflora	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Lactuca serriola	SCal	CH	KEELEY and KEELEY, 1987
Lagenifera huegelii	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Madia gracilis	SCal	CH	KEELEY and KEELEY, 1987
Metalasia densa (syn. *Metalasia muricata*)	CFR	CH S	PIERCE and MOLL, 1994 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Microseris linearifolia	SCal	CH	KEELEY and KEELEY, 1987
Millotia tenuifolia	WA/jf	SA/ssb	WARD, KOCH and GRANT, 1997
Myriocephalus stuartii	Aus	S	ROCHE, DIXON and PATE, 1997 BELL, 1999
Oedera capensis	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004

Species	Habitat	Treatment	Reference
Oncosiphon grandiflorum	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Oncosiphon suffruticosum	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Osteospermum fruiticosum (syn. *Dimorphotheca fruiticosa*)	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Othonna bulbosa	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Othonna quinquedentata	CFR	SW	BROWN, 1993a
Perezia microcephala	SCal	CH	KEELEY and KEELEY, 1987
Phaenocoma prolifera	CFR	S	BROWN and BOTHA, 2004
Plectostachys serphyllifolia	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Porophyllum gracile	SCal	CH	KEELEY and KEELEY, 1987
Pterochaeta paniculata	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Rhodanthe citrina	WA	SC SW	MERRITT, KRISTIANSEN, FLEMATTI, TURNER, GHISALBERTI, TRENGOVE and DIXON, 2006
Schoenia cassiniana	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Schoenia filifolia subsp. *subulifolia*	WA	SW	PLUMMER, ROGERS, TURNER and BELL, 2001
Senecio diaschides	WA/jf	SA/ssb	WARD, KOCH and GRANT, 1997
Senecio grandiflorus	CFR	SW	BROWN, 1993a
Senecio halimifolius	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Senecio hispidulus	WA/jf	SA/ssb	WARD, KOCH and GRANT, 1997
Senecio pinifolius	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Senecio rigidus	CFR	SW	BROWN, 1993a
Senecio umbellatus	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Stuartina muelleri	Aus	SW/ssb	ENRIGHT and KINTRUP, 2001
Syncarpha eximia	CFR	SA SW S	BROWN, 1993a BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Trichocline spathulata	WA/rm	SA SA/bcs	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999
Trypteris sinuata	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Ursinia sericea	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Ursinia tenuifolia	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Vigueira laciniata	SCal	CH	KEELEY, 1987
Vittadinia gracilis	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
BORAGINACEAE			
Cryptantha muricata	SCal	CH	KEELEY, 1984
BRASSICACEAE			
Brachycarpaea juncea	CFR	S	BROWN and BOTHA, 2004
Brassica nigra	SCal	CH	KEELEY, MORTON, PEDROSA and TROTTER, 1985
Descurainia pinnata	SCal	CH	KEELEY, MORTON, PEDROSA and TROTTER, 1985

Species	Habitat	Treatment	Reference
Heliophila coronopifolia	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Heliophila macowaniana	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Heliophila pinnata	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Sinapis arvensis		SW	ADKINS and PETERS, 2001 ADKINS, PETERS, PATERSON and NAVIE, 2003
Sisymbrium orientale	SCal	CH	KEELEY and KEELEY, 1987
BRUNIACEAE			
Berzelia abrotanoides	CFR	S	BROWN and BOTHA, 2004
Berzelia galpinii	CFR	S	BROWN and BOTHA, 2004
Berzelia lanuginosa	CFR	SW	BROWN, 1993a BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Brunia albiflora	CFR	S	BROWN and BOTHA, 2004
Staavia radiata	CFR	S	BROWN and BOTHA, 2004
CAMPANULACEAE			
Pratia purpurescens	NSW	SA/ssb	READ, BELLAIRS, MULLIGAN and LAMB, 2000
Wahlenbergia preissii	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
CAPRIFOLIACEAE			
Lonicera subspicata	SCal	CH	KEELEY, 1987
CARYOPHYLLACEAE			
Dianthus sp.	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Silene gallica	SCal	CH	KEELEY and KEELEY, 1987
CASUARINACEAE			
Allocasuarina fraseriana	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
CHLOANTHACEAE			
Lachnostachys eriobotrya	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Lachnostachys verbascifolia	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Pityrodia scabra	Aus	SA	ROCHE, DIXON and PATE, 1997
CISTACEAE			
Cistus creticus	MB/phr	CH	KEELEY and BAER-KEELEY, 1999
Cistus incanus	MB/phr	CH	KEELEY and BAER-KEELEY, 1999
Cistus monspeliensis	MB/phr MB	CH CE	KEELEY and BAER-KEELEY, 1999 PÉREZ-FERNÁNDEZ and RODRÍGUEZ-ECHEVERRÍA 2003
Cistus salvifolius	MB/phr	CH	KEELEY and BAER-KEELEY, 1999
Helianthemum scoparium	SCal	CH	KEELEY, MORTON, PEDROSA and TROTTER, 1985
CONVOLVULACEAE			
Dichondra repens	NSW	SA/ssb	READ, BELLAIRS, MULLIGAN and LAMB, 2000
CRASSULACEAE			
Crassula coccinea	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
CUCURBITACEAE			
Marah macrocarpus	SCal	CH	KEELEY, MORTON, PEDROSA and TROTTER, 1985

Species	Habitat	Treatment	Reference
DILLENIACEAE			
Hibbertia amplexicaulis	WA	SA SW	ROCHE, DIXON and PATE, 1997 BELL, 1999 ALLAN, ADKINS, PRESTON and BELLAIRS, 2004
Hibbertia commutata	WA WA/rm	SA SW/ssb SW	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999 ALLAN, ADKINS, PRESTON and BELLAIRS, 2004
Hibbertia quadricolor	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
DIPSACACEAE			
Scabiosa africana	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
DROSERACEAE			
Drosera gigantea	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Drosera macrantha	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Drosera pallida	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
EBENACEAE			
Diospyros glabra	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
EPACRIDACEAE			
Andersonia lehmanniana	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Astroloma ciliatum	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Astroloma foliosum	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Astroloma pallidum	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Leucopogon nutans	WA	SW	ALLAN, ADKINS, PRESTON and BELLAIRS, 2004
Leucopogon oxycedrus	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Leucopogon propinquus	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Leucopogon verticillatus	WA/jf WA/rm	CH SW/ssb SA/bcs	BELL, VLAHOS and WATSON, 1987 ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999
Styphelia tenuiflora	Aus WA/rm	SA SA/bcs	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 BELL, 1999
ERICACEAE			
Arctostaphylos viscida	SN	SW	KEELEY, McGINNIS and BOLLENS, 2005
Comarostaphylis diversifolia	SCal	CH	KEELEY, 1987
Erica arborea	MB	SA	CROSTI, LADD, DIXON and PIOTTO, 2006
Erica brachialis	CFR	SA	BROWN, KOTZE and BOTHA, 1993 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Erica capitata	CFR	SA	BROWN, KOTZE and BOTHA, 1993 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Erica cerinthoides	CFR	SA	BROWN, KOTZE and BOTHA, 1993 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Erica cruenta	CFR	SA	BROWN, KOTZE and BOTHA, 1993 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Erica dilatata	CFR	SA	BROWN, KOTZE and BOTHA, 1993

Species	Habitat	Treatment	Reference
Erica gallorum	CFR	SA	BROWN, KOTZE and BOTHA, 1993 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica glauca var. *elegans*	CFR	SA	BROWN, KOTZE and BOTHA, 1993 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica halicacaba	CFR	SA	BROWN, KOTZE and BOTHA, 1993 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Erica junonia var. *minor*	CFR	SA	BROWN, KOTZE and BOTHA, 1993 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Erica leptopus	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica longifolia	CFR	SW	BROWN, 1993a BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica oblongiflora	CFR	SA	BROWN, KOTZE and BOTHA, 1993 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Erica patersonii	CFR	SW	BROWN, 1993a BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Erica perspicua	CFR	S	BROWN and BOTHA, 2004
Erica peziza	CFR	SA	BROWN, KOTZE and BOTHA, 1993 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Erica pillansii	CFR	SA	BROWN, KOTZE and BOTHA, 1993 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Erica porteri	CFR	S	BROWN and BOTHA, 2004
Erica recta	CFR	SA	BROWN, KOTZE and BOTHA, 1993 BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Erica turgida	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Erica verecunda	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
EUPHORBIACEAE			
Mercurialis annua		SW	ADKINS and PETERS, 2001 ADKINS, PETERS, PATERSON and NAVIE, 2003
Phyllanthus calycinus	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Poranthera huegelii	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
FABACEAE (Papilionaceae)			
Bossiaea aquifloium	WA/jf	SA/ssb	WARD, KOCH and GRANT, 1997
Bossiaea ornata	WA/rm WA/jf	SW/ssb SA/ssb	ROCHE, KOCH and DIXON, 1997 WARD, KOCH and GRANT, 1997
Chamaecrista absus	Aus	SA	WILLIAMS, CONGDON, GRICE and CLARKE, 2003b
Chamaecrista mimoisoides	Aus	SA	WILLIAMS, CONGDON, GRICE and CLARKE, 2003b
Crotalaria calycina	Aus	SA	WILLIAMS, CONGDON, GRICE and CLARKE, 2003b
Crotalaria lanceolata	Aus	SA	WILLIAMS, CONGDON, GRICE and CLARKE, 2003b
Crotalaria montana	Aus	SA	WILLIAMS, CONGDON, GRICE and CLARKE, 2003b
Crotalaria pallida	Aus	SA	WILLIAMS, CONGDON, GRICE and CLARKE, 2003b
Cyclopia subternata	CFR	SW/scr	SUTCLIFFE and WHITEHEAD, 1995
Daviesia ulicifolia	NSW	SA/ssb	READ, BELLAIRS, MULLIGAN and LAMB, 2000
Galactia tenuiflora	Aus	SA	WILLIAMS, CONGDON, GRICE and CLARKE, 2003b
Glycine sp.	NSW	SA/ssb	READ, BELLAIRS, MULLIGAN and LAMB, 2000
Glycine tomentella	Aus	SA	WILLIAMS, CONGDON, GRICE and CLARKE, 2003b
Gompholobium knightianum	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997

Species	Habitat	Treatment	Reference
Gompholobium marginatum	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
Gompholobium preissii	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
Hovea pungens	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Hovea trisperma	WA/jf	SA/ssb	WARD, KOCH and GRANT, 1997
Indigofera filifolia	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Indigofera hirsuta	Aus	SA	WILLIAMS, CONGDON, GRICE and CLARKE, 2003b
Kennedia carinata	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
Kennedia coccinea	WA/jf	SA/ssb	WARD, KOCH and GRANT, 1997
Kennedia prostrata	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
Lotus salsuginosus	SCal	CH	KEELEY, 1984 KEELEY, MORTON, PEDROSA and TROTTER, 1985 KEELEY and KEELEY, 1987
Lotus scoparius	SCal	CH	KEELEY, 1987
Lotus strigosus	SCal	CH	KEELEY and KEELEY, 1987
Mirbelia dilatata	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
Otholobium fruticans	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Podalyria calyptrata	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Podalyria sericea	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Psoralea pinnata	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Pultenaea retusa	NSW	SA/ssb	READ, BELLAIRS, MULLIGAN and LAMB, 2000
Retama sphaerocarpa	MB	CE	PÉREZ-FERNÁNDEZ and RODRÍGUEZ-ECHEVERRÍA 2003
Sphaerolobium medium	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
Tephrosia juncea	Aus	SA	WILLIAMS, CONGDON, GRICE and CLARKE, 2003b
Trifolium angustifolium	MB	CE	PÉREZ-FERNÁNDEZ and RODRÍGUEZ-ECHEVERRÍA 2003
Virgilia divaricata	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
FAGACEAE			
Quercus dumosa	SCal	CH	KEELEY, 1987
GENTIANACEAE			
Chironia linoides subsp. *emarginata*	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
GERANIACEAE			
Pelargonium auritum	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Pelargonium capitatum	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Pelargonium cucullatum	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Pelargonium peltatum	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Pelargonium quercifolium	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Pelargonium sp.	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Pelargonium suburbanum	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004

Species	Habitat	Treatment	Reference
GOODENIACEAE			
Gonocarpus cordiger	Aus	SA	ROCHE, DIXON and PATE, 1997
Goodenia caerulea	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Lechenaultia biloba	WA/jf WA/rm	CH SW/ssb	BELL, VLAHOS and WATSON, 1987 ROCHE, KOCH and DIXON, 1997
Scaevola fasciculata	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Scaevola pilosa	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
GYROSTEMONACEAE			
Tersonia cyathiflora	Aus	S	ROCHE, DIXON and PATE, 1997 BELL, 1999
HALORAGACEAE			
Glischrocaryon aureum	WA/jf WA/rm	CH SW/ssb SA/bcs	BELL, VLAHOS and WATSON, 1987 ROCHE, KOCH and DIXON, 1997
HYDROPHYLLACEAE			
Eucrypta chrysanthemifolia	SCal	CH	KEELEY and KEELEY, 1987
Phacelia brachyloba	SCal	CH S	KEELEY, MORTON, PEDROSA and TROTTER, 1985 KEELEY and FOTHERINGHAM, 1998b
Phacelia parryi	SCal	CH	KEELEY, MORTON, PEDROSA and TROTTER, 1985
Phacelia viscida	SCal	CH	KEELEY, MORTON, PEDROSA and TROTTER, 1985
HYPERICACEAE			
Hypericum empetrifolium	MB/phr	CH	KEELEY and BAER-KEELEY, 1999
LAMIACEAE			
Hemiandra pungens	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Hemigenia ramosissima	WA/jf	SA/ssb	WARD, KOCH and GRANT, 1997
Hemiphora elderi	Aus	SA	ROCHE, DIXON and PATE, 1997
Lamium purpureum		SW	ADKINS and PETERS, 2001 ADKINS, PETERS, PATERSON and NAVIE, 200
Lamium purpureum		SW	ADKINS and PETERS, 2001 ADKINS, PETERS, PATERSON and NAVIE, 2003
Lavandula stoechas	MB	SA	CROSTI, LADD, DIXON and PIOTTO, 2006
Phlomis lanata	MB/phr	CH	KEELEY and BAER-KEELEY, 1999
Salvia apiana	SCal	CH	KEELEY, 1987
Teucrium sp.	MB/phr	CH	KEELEY and BAER-KEELEY, 1999
Trichostema lanatum	SCal	S	KEELEY and FOTHERINGHAM, 1998b
LOBELIACEAE			
Cyphia incisa	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Isotoma hypocrateriformis	WA/rm	SW/ssb SA/bcs	ROCHE, KOCH and DIXON, 1997
Lobelia pinifolia	CFR	S	BROWN and BOTHA, 2004
Lobelia rhombifolia	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
LOGANIACEAE			
Mitrasacme paradoxa (syn. *Phyllangium paradoxum*)	WA/jf	SA/ssb	WARD, KOCH and GRANT, 1997
MALVACEAE			
Althaea hirsuta	MB/phr	CH	KEELEY and BAER-KEELEY, 1999
Malacothamnus fremontii	SN	SW	KEELEY, McGINNIS and BOLLENS, 2005

Species	Habitat	Treatment	Reference
Malochothamnus fasciculatus	SCal	CH	KEELEY, 1987
MESEMBRYANTHEMACEAE			
Carpanthea pomeridiana	CFR	SW S	PIERCE, ESLER and COWLING, 1995 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Caryotophora skiatophytoides	CFR	acid, S SW S	HICKEY and VAN JAARSVELD, 1995 PIERCE, ESLER and COWLING, 1995 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Cleretum papulosum	SA	SW	PIERCE, ESLER and COWLING, 1995
Conicosia pugioniformis	CFR	SW S	PIERCE, ESLER and COWLING, 1995 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Drosanthemum bellum	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Drosanthemum bicolor	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Drosanthemum stokoei	CFR	SW S	PIERCE, ESLER and COWLING, 1995 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Erepsia aspera	CFR	SW	PIERCE, ESLER and COWLING, 1995 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Lampranthus austricolus	CFR	SW	PIERCE, ESLER and COWLING, 1995 BROWN and BOTHA, 2004
Lampranthus bicolor	CFR	SW	PIERCE, ESLER and COWLING, 1995 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Lampranthus promontorii	CFR	SW	PIERCE, ESLER and COWLING, 1995
Leipoldtia schultzei	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Oscularia deltoides	CFR	SW S	PIERCE, ESLER and COWLING, 1995 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Ruschia macowanii	CFR	SW S	PIERCE, ESLER and COWLING, 1995 BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Saphesia flaccida	CFR	acid, S	HICKEY and VAN JAARSVELD, 1995
Skiatophytum triplium	CFR	SW	PIERCE, ESLER and COWLING, 1995
MIMOSACEAE			
Acacia drummondii	WA/jf	SA/ssb	WARD, KOCH and GRANT, 1997
Acacia lateriticola	WA/jf	SA/ssb	WARD, KOCH and GRANT, 1997
Acacia pulchella	WA/jf	SA/ssb	WARD, KOCH and GRANT, 1997
MONTINACEAE			
Montinia caryophyllacea	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
MYOPORACEAE			
Myoporum turbinatum	Aus	S	ROCHE, DIXON and PATE, 1997 BELL, 1999
MYRTACEAE			
Calytrix flavescens	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Eucalyptus calophylla	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
Eucalyptus delegatensis	Aus	SA	CLOSE and WILSON, 2002
Eucalyptus marginata	WA/rm WA/jf	SA/ssb	ROCHE, KOCH and DIXON, 1997 WARD, KOCH and GRANT, 1997

Species	Habitat	Treatment	Reference
Eucalyptus regnans	Aus	SA	CLOSE and WILSON, 2002
Hypocalymma angustifolium	WA/jf WA/rm	CH SA/bcs	BELL, VLAHOS and WATSON, 1987 ROCHE, KOCH and DIXON, 1997
Hypocalymma robusta	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Hypocalymma robustum	WA/rm	SW/ssb SA/bcs	ROCHE, KOCH and DIXON, 1997
Sholtzia laxiflora	Aus	SA	ROCHE, DIXON and PATE, 1997
Thryptomene saxicola	Aus	SA	ROCHE, DIXON and PATE, 1997
OLEACEAE			
Fraxinus ornus	MB	SA	CROSTI, LADD, DIXON and PIOTTO, 2006
ONAGRACEAE			
Camissonia hirtella	SCal	CH	KEELEY, MORTON, PEDROSA and TROTTER, 1985
Camissonia unguiculata	SCal	CH	KEELEY and KEELEY, 1987
PAEONIACEAE			
Paeonia californica	SCal	CH	KEELEY, MORTON, PEDROSA and TROTTER, 1985
PAPAVERACEAE			
Dendromecon rigida	SCal	S	KEELEY and FOTHERINGHAM, 1998b
Dicentra chrysantha	SCal	CH S	KEELEY and KEELEY, 1987 KEELEY and FOTHERINGHAM, 1998b
Dicentra ochroleuca	SCal	CH	KEELEY and KEELEY, 1987
Papaver sp.	MB/phr	CH	KEELEY and BAER-KEELEY, 1999
PITTOSPORACEAE			
Billardiera bicolor	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Billardiera coeruleo-punctata	WA/rm	SW/ssb SA/bcs	ROCHE, KOCH and DIXON, 1997
Billardiera floribunda	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Billardiera variifolia	Aus	SA	ROCHE, DIXON and PATE, 1997
Billardiera variifolia	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
Bursaria spinosa	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Sollya heterophylla	WA/jf WA/rm	CH SW/ssb SA/bcs	BELL, VLAHOS and WATSON, 1987 ROCHE, KOCH and DIXON, 1997
PLANTAGINACEAE			
Plantago psyllium	MB/phr	CH	KEELEY and BAER-KEELEY, 1999
POLYGALACEAE			
Comesperma calymega	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Comesperma virgatum	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Muraltia squarrosa	Fynbos	CH	PIERCE and MOLL, 1994
POLYGONACEAE			
Chorizanthe fimbriata	SCal	CH	KEELEY and KEELEY, 1987
Eriogonum fasciculatum	SCal	CH	KEELEY, 1987
Polygonum aviculare		SW	ADKINS and PETERS, 2001 ADKINS, PETERS, PATERSON and NAVIE, 2003
Pterostegia drymarioides	SCal	CH	KEELEY and KEELEY, 1987
PROTEACEAE			
Adenanthos barbigerus	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
Aulax cancellata	CFR	S	BROWN, 1993a BROWN, VAN STADEN, DAWS and JOHNSON, 2003

Species	Habitat	Treatment	Reference
Aulax pallasia	CFR	S	BROWN and BOTHA, 2004
Aulax umbellata	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Banksia grandis	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
Conospermum triplinervium	Aus	S	ROCHE, DIXON and PATE, 1997 BELL, 1999
Dryandra carduacea	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Dryandra formosa	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Dryandra fraseri	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Dryandra nivea	WA/jf WA/rm	CH SA/bcs	BELL, VLAHOS and WATSON, 1987 ROCHE, KOCH and DIXON, 1997
Dryandra polycephala	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Dryandra sessilis	WA/jf WA/rm	CH SA/bcs	BELL, VLAHOS and WATSON, 1987 ROCHE, KOCH and DIXON, 1997
Grevillea pilulifera	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Grevillea pulchella	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Grevillea quercifolia	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Grevillea scapigera	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Hakea amplexicaulis	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Hakea cyclocarpa	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Hakea lissocarpha	WA/jf WA/rm	CH SA/bcs	BELL, VLAHOS and WATSON, 1987 ROCHE, KOCH and DIXON, 1997
Hakea ruscifolia	WA/rm	SW/ssb SA/bcs	ROCHE, KOCH and DIXON, 1997
Hakea stenocarpa	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Hakea trifurcata	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Hakea undulata	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
Isopogon dubius	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Leucadendron daphnoides	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Leucadendron dubium	CFR	S	BROWN and BOTHA, 2004
Leucadendron gandogeri	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Leucadendron glaberrimum	CFR	S	BROWN and BOTHA, 2004
Leucadendron laureolum	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Leucadendron linifolium	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Leucadendron muirii	CFR	S	BROWN and BOTHA, 2004
Leucadendron pubescens	CFR	S	BROWN and BOTHA, 2004
Leucadendron salicifolium	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Leucadendron sessile	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Leucadendron spissifolium subsp. *spissifolium*	CFR	S	BROWN and BOTHA, 2004
Leucadendron xanthoconus	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Leucospermum conocarpodendron	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Leucospermum cordifolium	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Leucospermum glabrum	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004

Species	Habitat	Treatment	Reference
Leucosperumum praecox	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Mimetes argenteus	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Mimetes cucullatus	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Paranomus reflexus	CFR	S	BROWN and BOTHA, 2004
Persoonia elliptica	Aus	SA	ROCHE, DIXON and PATE, 1997
Persoonia longifolia	Aus WA/rm	SA SW/ssb SA/bcs	ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997
Persoonia saccata	Aus	SA	ROCHE, DIXON and PATE, 1997
Petrophile linearis	Aus	SA	ROCHE, DIXON and PATE, 1997 BELL, 1999
Protea acuminata	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Protea cynaroides	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Protea eximia	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Protea longifolia	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Protea magnifica	CFR	SW S	BROWN, 1993a BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Protea obtusifolia	CFR	S	BROWN and BOTHA, 2004
Protea punctata	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Protea repens	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Serruria adscendens	CFR	S	BROWN and BOTHA, 2004
Serruria florida	CFR	SW S	BROWN, 1993a BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Serruria glomerata	CFR	S	BROWN and BOTHA, 2004
Spatalla curvifolia	CFR	S	BROWN and BOTHA, 2004
Synaphea petiolaris	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
RANUNCULACEAE			
Clematis flammula	MB	SA	CROSTI, LADD, DIXON and PIOTTO, 2006
Clematis pubescens	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Delphinium cardinale	SCal	CH	KEELEY and KEELEY, 1987
Delphinium parryi	SCal	CH	KEELEY and KEELEY, 1987
RHAMNACEAE			
Ceanothus cuneatus	SCal	CH	KEELEY, 1987
Ceanothus integerrimus	SCal	CH	KEELEY, 1987
Ceanothus leucodemis	SCal	CH	KEELEY, 1987
Ceanothus megacarpus	SCal	CH	KEELEY, 1987
Ceanothus oliganthus	SCal	CH	KEELEY, 1987
Cryptandra arbutifolia	Aus	SA	ROCHE, DIXON and PATE, 1997
Phylica buxifolia	CFR	S	BROWN and BOTHA, 2004
Phylica ericoides	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004

Species	Habitat	Treatment	Reference
Phylica pubescens	CFR	SA/SW	BROWN, 1993a BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Pomaderris obcordata	Aus	SA	ROCHE, DIXON and PATE, 1997
Rhamnus californica	SCal	CH	KEELEY, 1987
Trymalium ledifolium	WA/jf WA/rm	CH SA/bcs	BELL, VLAHOS and WATSON, 1987 ROCHE, KOCH and DIXON, 1997
Trymalium spathulatum	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
ROSACEAE			
Cercocarpus betuloides	SCal	CH	KEELEY, 1987
Heteromeles arbutifolia	SCal	CH	KEELEY, 1987
Sarcopoterium spinosum	MB/phr	CH	KEELEY and BAER-KEELEY, 1999
RUBIACEAE			
Anthospermum spathulatum	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003
Crucianella angustifolia	MB/phr	CH	KEELEY and BAER-KEELEY, 1999
Galium parisiense	SCal	CH	KEELEY and KEELEY, 1987
Opercularia echinocephala	WA/jf	SA/ssb	WARD, KOCH and GRANT, 1997
Opercularia hispidula	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
RUTACEAE			
Agathosma apiculata	Fynbos	CH	PIERCE and MOLL, 1994
Agathosma betulina	CFR	S	BROWN and BOTHA, 2004
Agathosma crenulata	CFR	S	BROWN and BOTHA, 2004
Agathosma stenopetala	Fynbos	CH	PIERCE and MOLL, 1994
Agathosma tabularis	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Boronia fastigiata	WA/jf WA/rm 	CH SA SA/bcs SA/ssb SW	BELL, VLAHOS and WATSON, 1987 ROCHE, DIXON and PATE, 1997 ROCHE, KOCH and DIXON, 1997 WARD, KOCH and GRANT, 1997 ADKINS, PETERS, PATERSON and NAVIE, 2003
Boronia megastigma	Aus	SA	ROCHE, DIXON and PATE, 1997
Boronia molloyae	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Boronia rosmarinifolia		SW	ADKINS, PETERS, PATERSON and NAVIE, 2003
Boronia spathulata	Aus	SA	ROCHE, DIXON and PATE, 1997
Coleonema album	CFR	S	BROWN and BOTHA, 2004
Correa reflexa	Aus	SA	ROCHE, DIXON and PATE, 1997
Diosma acmaeophylla	CFR	S	BROWN and BOTHA, 2004
Eriostemon spicatus	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
SCROPHULARIACEAE			
Antirrhinum nuttallianum	SCal	CH	KEELEY and KEELEY, 1987
Cordylanthus filifolius	SCal	CH	KEELEY and KEELEY, 1987
Keckiella antirrhinoides	SCal	CH	KEELEY, 1987
Keckiella cordifolia	SCal	CH	KEELEY, 1987
Keckiella ternata	SCal	CH	KEELEY, 1987
Mimulus aurantiacus	SCal	CH	KEELEY, 1987
Mimulus gracilipes	SN	SW	KEELEY, McGINNIS and BOLLENS, 2005
Misopates orontium	MB/phr	CH	KEELEY and BAER-KEELEY, 1999
Nemesia strumosa	CFR	S	BROWN and BOTHA, 2004

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Pseudoselago serrata	CFR	S	BROWN and BOTHA, 2004
Pseudoselago spuria	CFR	S	BROWN and BOTHA, 2004
Scrophularia californica	SCal	CH	KEELEY and KEELEY, 1987
Veronica hederifolia		SW	ADKINS and PETERS, 2001 ADKINS, PETERS, PATERSON and NAVIE, 2003
SOLANACEAE			
Anthocercis littorea	Aus	SA	ROCHE, DIXON and PATE, 1997
Solanum douglasii	SCal	CH	KEELEY and KEELEY, 1987
Solanum nigrum	NSW	SA/ssb	READ, BELLAIRS, MULLIGAN and LAMB, 2000
STACKHOUSIACEAE			
Stackhousia pubescens	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Stackhousia tryonii	WA	SW	BHATIA, NKANG, WALSH, BAKER, ASHWATH and MIDMORE, 2005
Tripterococcus brunonis	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
STERCULIACEAE			
Fremontodendron californicum	SCal SN	CH SW	KEELEY, 1987 KEELEY, McGINNIS and BOLLENS, 2005
Hermannia alnifolia	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Hermannia hyssopifolia	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Hermannia rudis	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Hermannia scabra	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Hermannia sp.	CFR	S	BROWN, VAN STADEN, DAWS and JOHNSON, 2003 BROWN and BOTHA, 2004
Lasiopetalum floribundum	WA/rm WA/jf	SW/ssb SA/ssb	ROCHE, KOCH and DIXON, 1997 WARD, KOCH and GRANT, 1997
Thomasia glutinosa	Aus	SA	ROCHE, DIXON and PATE, 1997
STYLIDIACEAE			
Levenhookia pusilla	WA/rm WA/jf	SW/ssb SA/bcs SA/ssb	ROCHE, KOCH and DIXON, 1997 WARD, KOCH and GRANT, 1997
Stylidium amoenum	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Stylidium bulbiferum	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Stylidium calcaratum	WA/rm WA/jf	SW/ssb SA/bcs SA/ssb	ROCHE, KOCH and DIXON, 1997 WARD, KOCH and GRANT, 1997
Stylidium hispidum	WA/rm	SW/ssb	ROCHE, KOCH and DIXON, 1997
Stylidium junceum	WA/rm WA/jf	SW/ssb SA/ssb	ROCHE, KOCH and DIXON, 1997 WARD, KOCH and GRANT, 1997
THYMELAEACEAE			
Passerina vulgaris	Fynbos	CH	PIERCE and MOLL, 1994
Pimelea ciliata	WA/rm	SW/ssb SA/bcs	ROCHE, KOCH and DIXON, 1997
Pimelea rosea	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Pimelea spectabilis	WA/jf WA/rm	CH SA/bcs	BELL, VLAHOS and WATSON, 1987 ROCHE, KOCH and DIXON, 1997
Pimelea suaveolens	WA/jf	CH	BELL, VLAHOS and WATSON, 1987
Pimelea sylvestris	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997

Species	Habitat	Treatment	Reference
VIOLACEAE			
Hybanthus calycinus	WA/rm	SA/bcs	ROCHE, KOCH and DIXON, 1997
Hybanthus floribundus	WA/jf	CH SA	BELL, VLAHOS and WATSON, 1987 ROCHE, DIXON and PATE, 1997 BELL, 1999