Fire-related germination cues for soil-stored seedbanks of fire-prone habitats in the Sydney region, Australia

Belinda J. Kenny

CERTIFICATE OF AUTHORSHIP / ORIGINALITY

I certify that this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree.

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ABSTRACT

Approximately 89% of species in fire-prone vegetation types of the Sydney region are assumed to have a soil seedbank. While a post-fire germination pulse is common for such species, the mechanisms involved in fire-related dormancy breaking are known for very few species in the region other than legumes.

The effects of the fire-related germination cues provided by soil heating and combustion products (smoke and charred wood) have been studied on numerous plant taxa in several regions of the world. However, these different cues have rarely been studied in combination.

The general aim of this thesis was to investigate the effect of fire-related germination cues on a variety of soil seedbank species of the Sydney region. This involved exploring methods of laboratory application of three fire-related cues (heat, smoke, and charred wood); assessing the individual and interactive effects of these cues on germination response (dormancy breaking) in laboratory, glasshouse and field trials; and examination of how these cues are received by soil-stored seeds.

Charred wood was found to have little effect on the germination of studied species, though the effects were very sensitive to the method of charred wood application. While half of the species studied were found to have a smoke cue, few of these responded to smoke only. Most species with only a smoke cue were sensitive to high temperatures (heat treatment was lethal or inhibitory).

One quarter of species had a heat cue only, most of these possessing a hard seed coat. A number of species without hard seed coats were also found to respond positively to heat, though these species also responded to smoke. The greatest proportion of studied species fell into the category of having germination stimulated by both heat and smoke. Among these species there was an even division into species with an equal germination response to both cues, an additive effect when the two cues are applied simultaneously, and a response only when the two cues are applied together.

The consequences of the different germination responses found are discussed in terms of the range of fire conditions likely to stimulate recruitment. Relationships were sought between functional types (fire response and seed traits) and these germination responses in order to explain the recruitment outcome of different fire regimes. It was found that the species showing a germination response that would allow germination under the widest range of fire conditions were those for which seedling recruitment is most critical after each fire event.

CHAPTER 1: INTRODUCTION
LITERATURE REVIEW AND STUDY AIMS

Post-fire Regeneration Methods

The dry sclerophyll vegetation of Australia is found under fire-prone climatic conditions, with high summer temperatures, periods of low rainfall and low humidity, and hot dry winds (Christensen et al. 1981). Under such conditions wildfires may start naturally (e.g. by lightning strike), or fires lit by man may get out of control (e.g. management, experiment, accident, or arson). This vegetation is highly flammable due to the low mineral nutrient content of, and the volatile oils contained in, the leaves of many species (Christensen 1985, Dickinson & Kirkpatrick 1985), and the rapid accumulation of fuel (van Loon 1977, Fox et al. 1979, Morrison et al. 1996). The combination of these factors allows for frequent and/or intense fire events.

Within fire-prone habitats, plant species have various strategies for maintaining the persistence of their populations. Plants can be broadly grouped depending on whether the majority of adults are killed by fire (obligate seeder species) or survive and regrow (resprouter species) (Gill 1981). Obligate seeders rely on reproductive regeneration for population persistence. Resprouters may show vegetative recovery only (obligate resprouters), or use both vegetative and reproductive regeneration (facultative resprouters) (Bell et al. 1984).

However, the popular view of a clear dichotomy between 'seeders' and 'resprouters' is simplistic (Gill et al. 2002, Whelan et al. 2002). Some species show a variable fire response, with variations arising from both environmental and genetic factors. The most obvious factor influencing fire response is the fire itself, with fire intensity having a significant influence on the damage sustained and hence ability to resprout (e.g. Morrison & Renwick 2000). For this reason, classification systems take this into account; such that "fire response" should refer to the response to complete burning (e.g. 100% canopy scorch of Gill 1981). Conditions that influence plant stress can affect post-fire regeneration, such as rainfall and temperature. Variation in mortality may also occur with plant age or size: young plants may need to acquire a certain height (Williams 1995), stem size (Morrison 1995), or bark thickness (Glasby et al. 1988); or develop structures such as lignotubers (Bradstock 1990) before fire tolerance is assured. Conversely, old plants may lose the ability to resprout (Burrows 1985). Variation in fire response has also been recorded within a species in different habitats (e.g. Benwell 1998) or populations (e.g. Rice & Westoby 1999, Pate et al. 1991).

Species can be further classed by other traits: the seedbank may be persistent (stored in serotinous cones, or dormant seeds stored in the soil) or transient (seeds released non-dormant to germinate immediately) (Thompson & Grime 1979); seed dispersal may be localised around the parent plant or over a wide enough area to allow recolonisation; seedling establishment may be intolerant (establish only after disturbance) or tolerant (establish both after disturbance or in mature communities) of competition (Noble & Slatyer 1980).

A variety of classification systems have been devised to describe plant fire responses (e.g. Tables 1.1-1.3). While these describe the response of a species to a single fire event, it is the interaction between these survival traits and the fire regime (frequency, intensity and season of fire) that will determine population persistence. The vital attributes scheme of Noble and Slatyer (1980) takes the classification of fire response further, by describing both persistence and establishment traits (Table 1.4) and then defining functional types that are the feasible combinations of these traits (Table 1.5). The fate of these functional types was then predicted under regimes of frequent and infrequent disturbance (Table 1.5).

Vegetative vs. Reproductive Regeneration

In the fire-prone dry sclerophyll vegetation of Australia, resprouters are more common than obligate seeders. In south-eastern Australia resprouters make up around 65% of species in dry sclerophyll forest (Purdie & Slatyer 1976) and 66-76% of dry heath species (Wark *et al.* 1987, Myerscough *et al.* 1995). Similarly, about 70% of species of Western Australian jarrah forests (Christensen & Kimber 1975, Bell & Koch 1980) and kwongan heath (Bell *et al.* 1984, van der Moezel *et al.* 1987) are resprouters. Wet sclerophyll communities (which experience less frequent fires) contain a larger proportion of obligate seeders, with only 40-50% of species resprouting (Christensen & Kimber 1975, Ashton 1981).

The interaction between fire regime (most importantly fire frequency) and regeneration method is an important determinant of community floristics and structure. Combinations of traits that render a species vulnerable to particular fire regimes are well documented (e.g. Noble & Slatyer 1980, Bond & van Wilgen 1996, Keith 1996, Keith *et al.* 2002, Bradstock & Kenny 2003). Species without vegetative recovery and with seedbanks that are either exhausted by a single fire or transient are most vulnerable to frequent fire. Inter-fire intervals shorter than the time required to produce new seed may result in local extinction. Species reliant on fire for recruitment are vulnerable to low fire frequency. Fire needs to occur before senescence of the standing population and degeneration of the seedbank (see Table 1.5).

Local decline or elimination of serotinous obligate seeder species (*Banksia ericifolia, Casuarina distyla, Hakea sericea, Hakea teretifolia* and *Petrophile pulchella*) has been observed in NSW coastal communities experiencing short inter-fire intervals (Siddiqi *et al.* 1976, Nieuwenhuis 1987, Morrison *et al.* 1995, Bradstock *et al.* 1997). Obligate seeders with soil-stored seedbanks may not be so adversely affected by frequent fires (Nieuwenhuis 1987); however this will depend on whether or not the entire seedbank is exhausted by a single fire event. The ability to retain a residual seedbank after a fire provides a buffer against the adverse effects of short fire intervals (Pausas 1999). A residual seedbank is most likely in species with a dose-related germination response to fire and a persistent seedbank, i.e. hard-seeded species which exhibit a depth-related response to heat (Auld & Tozer 1995). Cary & Morrison (1995) found leguminous obligate seeders to be relatively unaffected by short fire intervals compared to other soil-stored seeder species. While some obligate seeder species may manage to recolonise a burnt area from surrounding unburnt populations (Benson 1985), in general the seed dispersal mechanisms of these species are not well developed (Keeley 1986, Whelan 1986). As establishment of new seedlings tends to be linked to fire, obligate seeders may also be lost when the between-fire interval exceeds the species' endurance (plant life span plus seedbank longevity) at a site.

Table 1.1 A simple classification of woody plant species in relation to fire. From Gill (1981); loosely based on Raunkiaer's (1934) life-form classification.

Plants in the reproductive phase just subject to 100% leaf scorch by fire die (non-sprouters):
(1) Seed storage on plant
(2) Seed storage in soil
(3) No seed storage in burnt area
Plants in the reproductive phase just subject to 100% leaf scorch by fire recover (sprouters):
Subterranean regenerative buds:
(4) Root suckers, horizontal rhizomes
(5) Basal stem sprouts, vertical rhizomes
Aerial regenerative buds:
(6) Epicormic buds grow out
(7) Continued growth of active aerial pre-fire buds

Table 1.2 Methods of vegetative recovery by perennial shrub and herb species after burning. After Purdie (1977a).

Fire response	Vegetative recovery
(1) Fire-sensitive decreasers	No vegetative recovery
(2) Fire-resistant non-geophytic decreasers	Produce regrowth only from surviving rootstocks or tussocks
(3) Fire-resistant geophytic decreasers	Summer-dormant species producing vegetative shoots from bulbs
(4) Fire-resistant non-geophytic increasers	Produce regrowth and suckers from both rootstocks and lateral roots, can multiply vegetatively
(5) Fire-resistant geophytic increasers	Produce regrowth from rhizomes, can multiply vegetatively

Table 1.3 Categories of fire response seen in the kwongan vegetation of Western Australia. From Bell *et al.* (1984).

Fire response	Traits
(1) Fire ephemerals	Occur mainly in the first post-fire years, very short life span
(2) Obligate seeders	Growth cycle terminated by fire
(3) Sprouters	Self replacement (resprouting) occurs
(a) Obligate vegetatively-reproducing sprouter	Multiply by vegetative means, virtually no ability to produce seed
(b) Facultative sprouter-seeder	Limited self replacement, highly effective seedling recruitment
(c) Autoregenerating long-lived sprouter	High success at self replacement, limited seed production

Table 1.4 Vital attributes system of Noble & Slatyer (1980), describing attributes of persistence and establishment. Lifestage refers to the lifestage in which the method of persistence is available: J = juvenile, M = mature, **M** = mature tissue persists, P = propagule, E = locally extinct.

Persistence: Vital attribute		Persistence attributes	Lifestage
D		Propagules widely dispersed (hence always available)	JMPE
S		Propagules long lived, some remain after disturbance	JMP
G		Propagules long lived, exhausted after disturbance	MP
C		Propagules short lived	M
V		Resprout but lose reproductively mature tissue	JM
U		Resprout and rapidly reproductively mature	JM
W		Adults resprout (reproductively mature) but juveniles die	**M**
Combinations	**Act like**		
UD WD	Δ	Resprout, reproductively mature, propagules dispersed	JMPE
US WS UG	Σ	Resprout, reproductively mature, propagules stored	JMP
WG	Γ	Resprout, reproductively mature, propagules exhausted	MP
SD GD CD VD	D		
VS VG	S		
VC	V		
UC VW	U		
WC	W		
Establishment: Vital attribute	**Tolerance**		**Establishment**
I	Intolerant of competition		Establish and grow only after disturbance
T	Tolerant of a wide range of site conditions		Establish and grow both after disturbance and in mature community
R	Require conditions of mature community		Establish only in mature community

Table 1.5 Functional types and disturbance regimes resulting in extinction; after Noble and Slatyer (1980). Extinction may occur when intervals between disturbance events are less than the time to reproductive maturity (m) or greater than the life span (l) or life span plus seedbank longevity (l+e).

Group	Functional type	Disturbance regime resulting in local extinction
1	DT ST VT	none
2	GT CT	frequent (interval < m)
3	DI	none
4	SI	infrequent (interval > l+e)
5	GI	either (m > interval > l+e)
6	CI	either (m > interval > l)
7	VI	infrequent (interval > l)
8	DR SR	none
9	GR CR VR	first disturbance
10a	ΔT ΣT ΓT UT WT	none
10b	ΔR ΣR ΓR UR WR	none
11	ΔI	none
12	ΣI	infrequent (interval > l+e)
13	ΓI	either (m > interval > l+e)
14	UI WI	infrequent (interval > l)

With the ability for repeated rejuvenation of the canopy of adult plants, obligate resprouters (fire response vegetative only) are tolerant of both shorter and longer fire-free intervals than obligate seeders (Pausas 1999). Seedling recruitment does not occur directly after a fire and may require a fire-free period of reasonable length, as seeds are generally short-lived and fire intolerant. This ability for reproduction without fire is advantageous in areas of very low fire frequency (Keeley 1992a & b).

Facultative resprouters (fire response both vegetative and reproductive) are also reasonably tolerant of high fire frequency relative to obligate seeders (e.g. Bradstock *et al.* 1998a, Enright *et al.* 1998). Seedling recruitment, however, requires fire within the life span of adults or stored seed, as germination is usually stimulated by fire (Keeley 1986). While seedling recruitment is not necessary in every fire cycle, individual adults (lost to senescence or fire-induced mortality) will eventually need to be replaced in order to maintain a stable population. This requires enough time without fire for established seedlings to develop sufficient fire-resistant organs or buds (Benson 1985). This may take up to fifteen years, depending on the species, for example: 8 years for *Telopea speciosissima*, 7-9 years for *Banksia serrata*, 14-15 years for *Isopogon anemonifolius* (Bradstock & Auld 1987), and >8 years for *Angophora hispida* (Auld 1986a).

As seeder species are reliant on seedling recruitment alone for population persistence after a fire, seeder species tend to exhibit greater post-fire seedling establishment than resprouter species (Keeley 1977, Keeley & Zedler 1978, Moreno & Oechel 1992, Benwell 1998). This is achieved through differences in resource allocation, with resprouters concentrating resources towards survival and regrowth, while seeders need to ensure recruitment (Bond & van Wilgen 1996, Bellingham & Sparrow 2001).

There are many stages of the seedling establishment process to which obligate seeders may choose to allocate their resources; from seed production, through seed survival, to seed germination and emergence. Most studies have focused on the seed production stage, generally finding seeders to have greater numbers of flowers, higher seed to flower ratio, and greater overall seed production (see Bell 2001). Seed survival would depend on both predation levels and fire-induced mortality. Barro & Poth (1988) and Moreno & Oechel (1991) have shown that seeds of seeder species are more tolerant of increasing temperatures than those of resprouters, hypothesising that seeds of obligate seeders are better adapted to survive fire. Bell & Williams (1998) however did not find a consistent pattern of heat tolerance with fire response. For higher germination levels to occur it might be expected that seeders should have better viability levels and/or a stronger response to fire-related germination cues. Bell *et al.* (1995) and Roche *et al.* (1997a) found no correlation between seed viability and fire response, while Roche *et al.* (1997a) also found no fire response pattern in smoke-stimulated germination.

Reproductive Regeneration Methods
The post-fire environment is advantageous for seedling recruitment, as competition is reduced and available resources are increased (Tyler 1995). Germination of a wide range of species is thus timed to coincide with a fire: resprouters may be stimulated to flower immediately after fire, serotinous cones are opened by heat, and dormant soil-stored seeds may possess a fire-related germination cue (Bell *et al.* 1993).

The first flush of post-fire seed germination usually coincides with the first rainfall, and maximum germination occurs when seed release is timed well with rain (Bradstock & Myerscough 1981), hence the season of fire will influence the regeneration rate of seedlings. However, in the Sydney region there is not a strong seasonal pattern in average rainfall, while the yearly variation in monthly rainfall is high. Bradstock & Bedward (1992) predicted that the coincidence of fires with 'wet' years may be as influential as fire season on the long term population patterns in the area.

Post-fire seedling establishment is usually complete within the first year or two after the fire (Purdie & Slatyer 1976, Specht 1981, Keeley 1986, Wark *et al.* 1987, Auld & Tozer 1995). Seeds which do not germinate in this time may not germinate at all (Purdie 1977b), as the advantages of the post-fire environment diminish. The method of seed storage will influence the speed with which seedlings establish post-fire. Potentially the slowest to take advantage of the post-fire establishment conditions are post-fire flowering resprouters, as they need to resprout, flower, develop and release seeds which are then ready to germinate given adequate moisture. Species with canopy seed storage need to wait for the woody cones to open (the speed of this can depend on fire intensity) and release the seeds (which are also ready to germinate with adequate moisture). Soil-stored seeds with dormancy broken by the passage of fire need only to wait for adequate moisture, and thus are potentially the quickest to establish seedlings (Auld & Tozer 1995).

Post-fire Flowering

Many resprouters flower very rapidly after a fire in order to release seeds into the post-fire environment. These are often species with transient seedbanks, and as such this is their way of cueing seedling recruitment to fire. This response is more common in monocots (Keeley 1986), as their secondary juvenile period (the time taken to return to sexual reproduction) is generally shorter than that of dicots (Johnson *et al.* 1994). While many species have enhanced flowering in the few years following a fire (e.g. *Telopea speciosissima*; Pyke 1983), some species will flower abundantly only immediately after fire (e.g. *Xanthorrhoea* spp. and *Haemodorum* spp.; Baird 1977). Thus fire-enhanced flowering (termed pyrogenic flowering) may be either obligate or facultative.

Flowering can be triggered by many different factors which may be brought about by fire, such as: leaf removal (Gill & Ingwersen 1976), smoke (Keeley 1993), nutrient enhancement, increased light, decreased competition, changed temperature regime (Lamont & Runciman 1993), and changes in soil chemistry (Johnson *et al.* 1994).

Post-fire flowering is particularly common in species of *Xanthorrhoea*, many of which flower only rarely or not at all in the absence of fire (Specht *et al.* 1958, Gill & Ingwersen 1976). In *Xanthorrhoea australis*, burning not only increases the number of plants producing inflorescences, but also hastens the flowering process (Gill & Ingwersen 1976). In investigations of the primary cause of this response, Gill & Ingwersen (1976) found that both ethylene treatment and leaf clipping produced a flowering response in *X. australis*. Johnson *et al.* (1994) found that the pulse flowering pattern observed in *Blandfordia nobilis* populations was closely correlated to changes in soil chemistry in the post-fire environment. Most plants flowered prolifically only for the first three to four post-fire years, after which the soil returned to pre-fire conditions.

Seed Storage on Plant

Seed may be stored on a plant in woody fruits or capsules where the follicle or valve ruptures with exposure to high temperature, thus releasing the seeds after fire. The terms serotiny and bradyspory are used to describe this characteristic. While the word serotiny is used more often, bradyspory appears to be the more accurate term. This trait is common in Australian heath and South African fynbos communities (Keeley 1986).

Serotiny is particularly common in species of the Proteaceae, Myrtaceae, and Casuarinaceae families (Lamont *et al.* 1991). The seeds of *Banksia ornata* are formed in woody follicles with valves held closed by a resin (Wardrop 1983). The valves are opened by fire as this resin melts. Without fire, the majority of follicles (about 98%) remain closed until the parent plant dies (Gill & McMahon 1986). Those follicles which manage to open without fire or plant death are most likely to be on cones near the base of the plant (Bradstock & Myerscough 1981, Gill 1981).

Seed Storage in Soil

The seeds of most plants in fire-prone environments are released from the parent plant on maturity (Whelan 1986). If this seed output does not remain viable for more than one year, the seedbank is considered to be transient. Such seed rarely becomes buried in the soil profile, germinating as soon as the right seasonal conditions occur. Seed output that does not germinate immediately and remains viable for periods greater than one year forms a persistent seedbank which becomes buried within the soil profile. To maintain a persistent soil-stored seedbank some form of seed dormancy is required (Thompson & Grime 1979).

In environments that require disturbance for successful seedling establishment, the cuing of germination from a persistent seedbank allows temporal dispersal of seeds to sites where resources are available (Parker *et al.* 1989). In fire-prone habitats, where this periodical establishment is linked to fire, the seed needs to receive a cue that indicates the passage of fire. Thompson (1978) considered accumulation of a soil seedbank to be a viable strategy only under high disturbance rates. Approximately 89% of plants in fire-prone vegetation of the Sydney region have persistent soil seedbanks (Auld *et al.* 2000).

The level of post-fire emergence from persistent seedbanks depends on the longevity, size and distribution of the seedbank, as well as germination cues received and environmental conditions at the time (Auld *et al.* 2000). Vertical distribution of the soil seedbank is of particular importance with respect to fire. Seeds within the litter layer or close to the soil surface may be destroyed by combustion (Borchert & Odion 1995), while seeds buried too deep may either not receive adequate stimulation (Auld & O'Connell 1991) or may not be able to successfully emerge (Bond *et al.* 1999).

Mechanisms of Seed Dormancy

All seeds have a range of conditions (water, temperature, light, and atmosphere) over which germination will occur. A dormant seed (sometimes referred to as refractory), however, will not germinate within these conditions until the dormant state is broken (Mayer & Poljakoff-Mayber 1989). Villiers (1972) gave a concise definition of this:

- Dormancy is a "state of arrested development whereby the organ, by virtue of its structure or chemical composition, may possess one or more mechanisms preventing its own germination";

- Quiescence is a "state of arrested development maintained solely by unfavourable environmental conditions".

A seed may be in the dormant state at the time of dispersal (primary dormancy), while non-dormant seeds that do not encounter favourable germination conditions may either remain quiescent or be induced into dormancy by subsequent conditions (secondary dormancy). Conversely, dormancy may be relaxed over time, through reversal of impermeability (Morrison *et al.* 1992) or 'after-ripening' (Mayer & Poljakoff-Mayber 1989).

There are three broad classes of dormancy, which may occur singularly or in combination (Baskin & Baskin 1989):

- Physical Dormancy   Imposed by characteristics of the seed coat

- Physiological Dormancy   Germination inhibiting mechanism in the embryo

- Morphological Dormancy   Underdeveloped embryo

Dormancy allows a seed to choose when it germinates, either avoiding conditions detrimental to seedling survival, or taking advantage of optimal conditions (e.g. a disturbed environment). Thus dormancy breaking mechanisms are related to the preferred establishment conditions, e.g. ambient temperature fluctuations signal the season, light levels indicate the depth of burial, and fire-related cues signal the passage of fire (Bell 1999).

Physical Dormancy

The most common form of physical dormancy is a seed coat that is impermeable to water. By blocking water entry the seed cannot imbibe and hence cannot commence germination (Ballard 1973). The hard seed coat needs to be softened or punctured by means such as scarification (physical abrasion), passing through an animal's digestive tract, fluctuating temperatures, high temperature or high humidity (Rolston 1978). Legumes are the best known hard-seeded plants. Permeability in legumes appears to be under the control of the strophiole (Hagon & Ballard 1969). Heat acts to open the strophiole by splitting the thin-walled cells beneath the palisade cells (Hanna 1984).

The seed coat may also impose dormancy through oxygen impermeability, prevention of radicle extension, trapping endogenous inhibitors within the embryo, or containing inhibitors itself (Kelly *et al.* 1992).

Physiological Dormancy

Vleeshouwers *et al.* (1995) regard physiological dormancy as "the seeds fastidiousness about the germination conditions it requires", such that the range of conditions in which germination will occur is widened by dormancy relieving factors. Factors that do not alter this fastidiousness yet are still necessary for the germination response are merely germination inducing factors.

Physiological dormancy is regulated by endogenous hormonal control via the balance of growth inhibitors and promoters. As the seed matures there is either a decrease in promoters or an increase in inhibitors, inducing dormancy. A trigger is then required to stimulate hormone activation or hormone synthesis to counteract this. A dormancy relieving trigger is necessary only to break dormancy, its continued presence during germination is not required (Amen 1968). Triggers may be photochemical reactions, thermochemical reactions (e.g. after-ripening, stratification), or removal of inhibitors (e.g. scarification, leaching).

Fire-Related Breaking of Dormancy

The most thoroughly studied fire-related germination cue is heat. High temperatures endured during the passage of a fire act to break physical dormancy. Fire may also act as a germination cue by removing inhibitory chemicals produced by other plants or micro-organisms, changing the environmental conditions of the soil profile (e.g. increased availability of nutrients, changed temperature and light regimes), or by triggering a physiological dormancy-relieving mechanism. Leachates from charred wood have been demonstrated to promote germination of some Californian chaparral species, and smoke has more recently been discovered to enhance germination in various South African and Western Australian species.

Heat

During the passage of a fire, temperatures at the soil surface are extreme, however due to the porous nature of soil, only a fraction of this heat is transferred into the soil profile, and heating is rapidly attenuated with depth (DeBano *et al.* 1979). Within the top few centimetres of the soil profile temperatures reached tend to be within the range of 50-150 °C in various fire-prone habitats: woodland, open forest and wet sclerophyll forest of south-eastern Australia (Beadle 1940, Floyd 1966, Bradstock & Auld 1995), semi-arid mallee of eastern Australia (Bradstock *et al.* 1992), jarrah forest of Western Australia (Smith *et al.* 2000) and Californian chaparral (Odion & Davis 2000).

The actual level of heating encountered by buried seeds will vary spatially with factors such as the fire intensity and duration, vegetation and litter cover (Atkins & Hobbs 1995, Bradstock & Auld 1995), soil texture and moisture (Beadle 1940, DeBano *et al.* 1979), as well as the depth of seed burial. Seeds within the litter layer or close to the soil surface may be destroyed by combustion (Borchert & Odion 1995), while seeds buried too deep may not receive adequate stimulation (Auld & O'Connell 1991). Thus there is a complex interaction between the spatial variability of the actual fire and soil heating, the vertical distribution of the seedbank, the thermal tolerance of the seed, and the temperature required to break seed dormancy.

Seeds from various families (most notably leguminous species) have physical dormancy imposed by a water-impermeable ('hard') seed coat. Dormancy is broken by a heat-pulse cue, as disruption of the seed coat allows imbibition and germination (Cavanagh 1987). Most studies of heat-shock as a germination cue have focused on hard-seeded species. A few recent studies have shown heat to stimulate germination of other seed morphologies (Morris 2000, Tieu *et al.* 2001a), warranting further investigation of the role of heat in species with seed coat dormancy mechanisms other than water-impermeability (Brits *et al.* 1993, Morris *et al.* 2000). Brits *et al.* (1999) have found that heat leads to scarification through desiccation in *Leucospermum* (Proteaceae) species with oxygen-impermeable seed coats.

Temperatures required for breaking hard seed coats have been shown to fall within the range 60-120 °C (with a peak between 80-100 °C) in species from a wide range of fire-prone habitats: heath and open forest of south-eastern Australia (Auld & O'Connell 1991), jarrah forest of Western Australia (Shea *et al.* 1979), Californian chaparral (Keeley *et al.* 1981), forests of south-eastern USA (Martin *et al.* 1975), South African fynbos (Cocks & Stock 1997), and garrigue of southern France (Trabaud & Oustric 1989). Optimal temperature for germination of hard-seeded species has been found to vary between co-existing species (Trabaud & Oustric 1989, Auld & O'Connell 1991, Atkins & Hobbs 1995, Cocks & Stock 1997).

Fire intensity, through its effect on heat-stimulated germination, has the ability to influence species composition in regenerating communities. Legume species have been shown to germinate more prolifically after high-intensity fires (Christenson & Kimber 1975, Auld 1986b, Auld & O'Connell 1991), and are at risk of decline under regimes of recurrent low intensity fires (Auld & O'Connell 1991, Keith 1996). On the other hand, heat-sensitive species germinate more prolifically after lower-intensity fire (Moreno & Oechel 1991, Tyler 1995).

Interactions between temperature and duration of heating (the 'heat sum') have been found (Keeley *et al.* 1985, Auld & O'Connell 1991, Cocks & Stock 1997, Tieu *et al.* 2001a). However, in terms of optimal temperature for dormancy-breaking, the effect of actual temperature is far greater than the effect of duration (Auld & O'Connell 1991). Duration of heating is a more critical factor for heat-induced seed mortality. At temperatures higher than the optimal for dormancy-breaking, seed mortality begins to occur. While short durations (c. 5 minutes) at high temperatures (c. 120 °C) can be endured, mortality rapidly increases at greater durations (c. 30 minutes) (Auld & O'Connell 1991, Cocks & Stock 1997). Heat tolerance in seeds without a hard seed coat is greatly reduced when in the imbibed state (Beadle 1940, Sweeney 1956). This has implications for seed survival in fires conducted under moist conditions (i.e. winter prescribed burns; Borchert & Odion 1995).

Barro & Poth (1988) and Moreno & Oechel (1991) have shown that seeds of seeder species are more tolerant of increasing temperatures than those of resprouters, hypothesising that seeds of obligate seeders are better adapted to survive fire. Bell & Williams (1998) however did not find a consistent pattern of heat tolerance with fire response.

Attempts have also been made to relate heat tolerance to seed size. Generally, larger seeds have been found to be tolerant of higher temperatures than small seeds (Valbuena *et al.* 1992, Gonzalez-Rabanal & Casal 1995, Gashaw & Michelsen 2002); however Hanley *et al.* (2003) have recently reported the opposite effect. These discrepancies probably arise due to the complication of the temperature-duration

factor. Large seeds may tolerate short bursts of high temperatures better than small seeds, but long durations of lower temperatures are more lethal to large seeds (Keeley 1991). Obviously there is more to heat tolerance than simply seed size, for example the surface to volume ratio (Keeley 1977). Cocks & Stock (1997) have found that the thicker the seed coat is relative to the embryo, the higher the temperature and heating duration required for optimal germination.

Combustion Products

In a comprehensive study of fire effects on herbaceous chaparral plants, Sweeney (1956) concluded from field observations that species with refractory seeds were stimulated to germinate by fire. In germination experiments, however, neither heat nor ash treatment gave any germination response in these species, although scarification did. Sweeney found "no reason to assume that ash is an important factor in increasing germination or breaking dormancy of seeds of herbaceous plants occurring on burns." It is unfortunate that he took these experiments no further, as one of his test species, *Emmenanthe penduliflora*, has subsequently been found to respond to both charred wood and smoke.

Charred Wood

The first report of a combustion product aiding in a seed's germination was that of Wicklow (1977) where he stated that "*Emmenanthe pennduliflora* Benth., a post-fire chaparral annual, has been shown in laboratory experiments to incorporate a unique mechanism allowing for the germination of its seeds." This species is one of several chaparral species that Sweeney (1956) deemed to germinate in response to fire, but could give no explanation as to the mechanism by which this occurred. Wicklow (1977) was determined to find the trigger, and performed experiments where unburned, charred or ashed *Adenostoma fasciculatum* (a chaparral shrub) branches were tested as the agent. Only the charred wood treatment resulted in germination. He thus found his unique germination trigger, and hesitantly suggested its action was via inactivation of an inhibitor within the seed.

Jones & Schlesinger (1980) supported Wicklow's results when they repeated his experiments on *E. penduliflora*. While they found the chaparral population of the species to respond to a charred wood treatment, seeds from the desert population (which rarely experiences fire) gave little reaction, relying mainly on physical scarification. They did not agree with Wicklow's thoughts on the mechanism of charred wood's action, but neither did they hypothesise an alternative.

Keeley & Nitzberg (1984) found that both *E. penduliflora* and *Eriophyllum confertiflorum* react to charate (powdered charred wood), though the magnitude of response was dependent on the medium on which the seeds were sown and moisture level. Heated (but not charred) wood was also effective, as was heated soil, and inhibition of *E. confertiflorum* germination from soil extract was overcome by adding charate. They hypothesised that either charate deactivates or binds an inhibitor in the soil, or charate acts on seeds by affecting an inhibitor, affecting permeability of the seed coat membrane (though no visible changes to the seed coat were observed) or some other stimulatory means. The active ingredient was water soluble, and was not activated charcoal. Work in the boreal forests of Sweden (Zackrisson *et al.* 1996, Wardle *et al.* 1998) supports the first hypothesis, as charcoal has been shown to adsorb phenolics produced by the vegetation as well as allow greater nutrient uptake by plants. These effects had a positive influence on the growth of seedlings, though no germination experiments were performed.

Keeley then tested a wide range of herbaceous (Keeley *et al.* 1985) and woody (Keeley 1987) chaparral species. Some species responded to charate, some to heat and a couple (including *E. confertiflorum*) with a synergistic effect of charate and heat. Partially charred wood was found to be as effective as fully charred, and the amount of charate used was not critical; a large variation in amount of charate used gave the same germination level. Comparison of charate with Hoagland's solution showed that charate enhancement is not due to a fertilisation effect.

Keeley & Pizzorno (1986) then began investigating the mode of charate action by testing different woods and wood products. They concluded that the active ingredient is derived from the hemicellulose part of the wood, most likely resulting from a thermal change in xylan. The thermal breakdown of xylan may release oligosaccharins which can act as regulators of many plant hormones (Albersheim & Darvill 1985). Keeley again found here that the active ingredient is water soluble, but treated seeds are not deactivated by later rinsing. This would indicate that contact with the ingredient begins a process within the seed which then continues of its own accord (i.e. breaks a physiological dormancy).

Little has been done with charred wood outside of the Californian chaparral. Brown (1993a) was the first to report a fynbos species (*Syncarpha vestita*) to be stimulated by charred wood. The promotive effective of charred wood was very similar to that of smoke derived from the same plant. This observation has since been supported for a wider range of fynbos species (Keeley & Bond 1997). In Australia very few experiments have been tried, and with little success. Bell *et al.* (1987) found only two out of forty jarrah understorey species tested with a significant positive charred wood enhancement. Marsden-Smedley *et al.* (1997) got a positive response to charred wood alone from only one exotic herb. Enright & Kintrup (2001) found no charred wood effect on either seedling density or species richness emerging from treated soil. Charred wood has also proven ineffective in the phrygana communities of Greece (Keeley & Baer-Keeley 1999).

Ash

Ash results from a more complete combustion process than charred wood. Byram (1959) describes the three phases of combustion: (i) pre-heating and drying of fuel; (ii) ignition and combustion of fuel, leaving charcoal; and (iii) combustion of charcoal, leaving ash. The chemical and physical properties, and therefore effects, of ash are quite different to those of charcoal. Unfortunately reports of the effects of ash can be hard to interpret as it is often not specified what level of combustion was achieved. However it appears that ash is more likely to inhibit the germination of non-dormant seeds, than have a stimulatory effect on dormant seeds.

Enright *et al.* (1997) found that an ash treatment applied over soil increased the soil's pH, exchangeable cations and extractable phosphorus, and resulted in the lowest seedling density of all applied treatments. Facelli & Kerrigan (1996) also found ash spread over soil to inhibit seedling establishment, as well as increase the mortality rate of the seedlings that did emerge. In the pine forests of Israel, germination of all species except *Rhus coriaria* is inhibited by ash cover. Even for *R. coriaria*, where thin ash cover (1-2 cm) encourages germination, germination is inhibited by thicker ash cover (5 cm) due to the resultant high pH and low water potential (Ne'eman *et al.* 1999).

Ash supplied either dry or in suspension to seeds in Petri dishes has also resulted in inhibition of germination. Sweeney (1956) found that ash reduced the germination response of sixteen herbaceous chaparral species. Gonzalez-Rabanal & Casal (1995) tested several species of the Ericaceae, Cistaceae and Poaceae, finding all species inhibited by the ash treatment. They also cite other Spanish research showing ash inhibition (Pereiras 1984, Trabaud & Casal 1989; cited in Gonzalez-Rabanal & Casal 1995).

Negative effects of ash on germination have also been reported for several conifer species (Thomas & Wein 1985, Thomas & Wein 1990, Neeman et al. 1993a, Neeman et al. 1993b, Reyes & Casal 1998) but this toxicity is removed by leaching of the ash. Thomas & Wein (1994) estimate that sufficient leaching by rain in field conditions would take up to three years post-fire.

Smoke

Research into smoke-stimulated germination is a relatively new field, with the majority of work so far being conducted in South Africa, Western Australia, and California. Following on from thoughts about nutrient enrichment from ash, van de Venter & Esterhuizen (1988) postulated that gases released in fires may be responsible for stimulation of germination. They exposed the seeds of two fynbos *Erica* species to heat, ethylene and ammonia, these two gases being known germination stimulators and produced during vegetation fires. While one species failed to respond to any treatment, the other had a small positive response to all three.

This idea has since been followed in a more general way by direct application of plant-derived smoke. While a wide range of species have been found to respond to a smoke treatment, the active ingredient has remained elusive, though it appears to be water soluble (i.e. aqueous extracts are also effective; Baxter *et al.* 1994, Baldwin *et al.* 1994, Jager *et al.* 1996a). It has been suggested that there are several active chemicals involved (Baldwin *et al.*1994, van Staden *et al.* 1995a).

Areas Studied

de Lange & Boucher (1990) initiated the smoke application approach in studies of the threatened fynbos species *Audouinia capitata*. Plant material was burnt in a drum and the smoke produced blown into a tent covering a patch of ground within the study species' habitat. A high level of germination occurred in these treated patches compared to none in untreated areas.

Brown continued the work on South African fynbos species, finding positive smoke reactions in species of Asteraceae, Ericaceae, Restionaceae, and Proteaceae (Brown 1993a & b, Brown *et al.* 1993, Brown *et al.* 1994, Brown *et al.* 1998). Many smoke-treated species of *Erica* (Brown *et al.* 1993b) and Restionaceae (Brown *et al.* 1994) gave a germination increase of three orders of magnitude compared to controls. It was suggested that those species with a lesser response to smoke treatment may respond to multiple cues. For example, the Restionaceae *Staberoha distichya* in which smoke treatment improved germination (Brown 1993b) had previously been shown to respond to heat treatment (Musil & de Witt 1991).

Most American research in this field has concentrated on charred wood rather than smoke. Initially only a few species from the pinyon-juniper, sagebrush (Baldwin *et al.* 1994, Baldwin & Morse 1994, Blank & Young 1998) and chaparral communities were tested (Keeley & Fotheringham 1997). The chaparral

annual *Emmanthe penduliflora* has received a lot of attention in the past in charred wood experiments. The 100% germination achieved with smoke treatment (Keeley & Fotheringham 1997) exceeds the effects of charred wood, which has given maximum germination ranging from 20-89% in various experiments (Wicklow 1977, Jones & Schlesinger 1980, Keeley & Nitzberg 1984, Keeley *et al.* 1985, Keeley & Pizzorno 1986, Thanos & Rundel 1995).

With the similarities shown between the habitats and species responses of the South African fynbos and Californian chaparral, Keeley & Bond (1997) combined to look at germination of species from both areas. As most chaparral work had concentrated on charred wood and fynbos work on smoke, they used both treatments on all seeds. They found that species responding to one treatment would respond in a similar manner to the other.

In Australia the technique of smoke treatment was first used by researchers at the Western Australian Kings Park and Botanic Garden. The initial experiments of Dixon *et al.* (1995) involved exposing seeds of 94 native Western Australian species considered to be difficult to germinate to aerosol smoke treatment, 45 of which showed a positive reaction. They also conducted field experiments exposing bushland sites to aerosol smoke, smoked water and smoked sand treatments. Again, many species showed improved germination with these treatments. Even a site which had already experienced a cool burn showed many species with increased recruitment following additional smoke treatment. They have since induced more species to successful germination with smoke treatment (Roche *et al.* 1994, Roche *et al.* 1997a) and are using this research to aid in mine-site rehabilitation (Roche *et al.* 1997b, Grant & Koch 1997, Ward *et al.* 1997).

Little research has yet been done elsewhere in Australia. Experiments using smoke as one of several treatments have been conducted on soil samples in Tasmania (Marsden-Smedley *et al.* 1997), Victoria (Enright *et al.* 1997, Enright & Kintrup 2001, Wills & Read 2002) and New South Wales (Read *et al.* 2000), but there have been few germination trials conducted on individual species. Keith (1997) found that both heat and smoke enhance the germination of the endangered Tasmanian *Epacris stuartii*. The treatment effects were equal in magnitude and additive, such that smoke and heat applied together produced the best result.

Few species in New South Wales have been shown to have a smoke response. Clarke *et al.* (2000) examined the germination of 65 species from the New England Tablelands, finding only one species with smoke-stimulated germination. In the same region, Grant & MacGregor (2001) found only four of 69 species with an apparent smoke response. However, several native grass species have been shown to respond favourably to smoke treatment (Read & Bellairs 1999, Read *et al.* 2000), and Morris (2000) has shown a positive smoke response in seven Sydney *Grevillea* species. More work is needed to see if smoke is an important germination cue in the fire-prone vegetation of New South Wales.

Active Ingredient

The initial smoke cue research of de Lange & Boucher (1990) showed that the active smoke agent/s can be chemically extracted. However, they concluded that the active ingredient was unlikely to be ethylene as had been suggested by van de Venter & Esterhuizen (1988). Baxter *et al.* (1994) concluded that neither ethylene nor ethrel (an ethylene producing compound) was responsible for the smoke induced

germination of the African and Australian grass *Themeda triandra*, proposing that a thermal breakdown product of hemicellulose or cellulose may be the cause. They found that the bioactive compound is also obtained from simply dry heating the same plant material as was burnt to produce the smoke. Jager *et al.* (1996a) also found this, and investigated the range of temperatures over which this occurred. Aqueous solutions of various concentrations were prepared from *Themeda triandra* leaves heated over a range of temperatures. Those heated at 180 °C and 200 °C had similar stimulatory effects on germination as smoke extract, while the extreme temperatures tested (140 °C and 240 °C) had no effect. Chromatography showed that the smoke and heat-derived extracts had similar chemical properties.

Keeley & Fotheringham (1997, 1998a & b) have also eliminated ethylene as the active ingredient in smoke, along with nitrate ion, nitrous oxide, carbon dioxide, and methane. They have found a strong response to nitrogen oxides, consistent with levels found in natural biomass smoke. These experiments have been performed on deeply dormant seeds of fire-prone environments, and hence probably have more ecological meaning then the lettuce bioassay described below.

In their search for the active ingredient in smoke a group of South African researchers have developed a bioassay with Grand Rapids lettuce (*Lactuca sativa* L. cv. Grand Rapids) as it responds to smoke extracts at a range of concentrations, germinating rapidly (Drewes *et al.* 1995). In experiments applying both smoke and various hormones, they also ruled out ethylene as the active smoke ingredient (van Staden *et al.* 1995b). Ethylene has previously been shown to have no effect on dormant seeds of this lettuce species, although it does increase germination in freshly imbibed seeds (Abeles & Lonski 1969). Jager *et al.* (1996b) again found neither ethylene nor octanic acid to be responsible for smoke-related breaking of lettuce seed dormancy. Germination was found to be dependent on the concentration of the applied smoke extract, with the highest concentrations being inhibitory. They have identified twelve compounds from smoke, seven of which were common to the two different species used to produce the smoke, but have so far not determined the active ingredient/s (van Staden *et al.* 1995a & c). The same active compound/s obtained from burning plant material were also present in agar and cellulose (Baldwin *et al.* 1994, Jager *et al.* 1996a), as well as in a commercial smoke food flavourant (Baldwin *et al.* 1994, Jager *et al.* 1996c). Adriansz *et al.* (2000) have isolated one possible component of smoke, 1,8-cineole, as a potential germination cue.

Action of Smoke

While the action of smoke remains unknown, the hypotheses are many and varied, though it should be remembered that the test species and their dormancy mechanisms have also been varied. The suggestions outlined below imply that smoke is acting on physiological dormancy mechanisms in some species, and on physical dormancy in others.

Pierce *et al.* (1995) suggest that smoke acts against a general germination inhibitor, as they found both fire-prone and non-fire-prone South African succulents reacted to smoke treatment. Baxter *et al.* (1994) found that the stimulatory effect of smoke treatment was related to the seed's imbibition, and proposed that smoke's action is through either an enzyme system or phytochrome metabolism. Baldwin *et al.* (1994) also suggest that germination stimulation works via a smoke-specific signal molecule, with smoke treatment stimulating metabolic activity of dormant seeds. They found no evidence to support their

alternative hypotheses of action via scarification or nutritive stimulation. Keeley & Fotheringham (1997) hypothesise that smoke and NO_2 treatments act on dormant *E. penduliflora* seeds by increasing the permeability of the subdermal cuticle to solutes. van Staden *et al.* (1995b) found an interaction between smoke and gibberellins (GA_3) in the germination of light-sensitive Grand Rapids lettuce, indicating that smoke caused an increase in sensitivity to ABA. They concluded that rather than working via phytochrome effects, smoke was promoting germination of dormant seeds by altering either membrane function or hormone receptor sensitivity. In a further study they found cytokinins (BA) to be more effective than gibberellins (Strydom *et al.* 1996). In experiments with celery, Thomas & van Staden (1995) found smoke extract to act on dormant seeds in a manner similar to ethephon and cytokinin, and suggest that its action is to enhance gibberellin activity within the seed's system.

de Lange & Boucher (1993) found that a characteristic fracture of the pericarp occurred during germination of smoke treated *A. capitata* seeds. Work by Keeley & Fotheringham (1998a & b) has also shown that the seed coat may be involved. Amongst a group of 25 smoke-stimulated chaparral species, most germinated well following mechanical scarification, and some after chemical scarification. These seeds, when untreated, will readily imbibe water but remain dormant. When water uptake was examined with dyes, while water was readily absorbed by the testa, it was stopped by a subdermal barrier before reaching the endosperm. Smoke treatment, however, allowed penetration through to the embryo. Egerton-Warburton (1998) has examined cuticle changes to one of these species, *Emmenanthe penduliflora*. After smoke treatment, changes on the seed's water-permeable external surface indicated that intense chemical scarification had occurred. Smoke also increased the density and width of permeate channels within the semi-permeable internal (sub-testa) cuticle. This evidence may indicate the presence of inhibiting agents within the endosperm which are too large to diffuse through the water-permeable cuticle. Scarification and the effects of smoke treatment may allow the outward passage of these.

Once pre-treated with smoke, seeds can be dried and stored, retaining the benefits of smoke treatment for later germination (Baxter & van Staden 1994, Brown *et al.* 1998). While Baldwin *et al.* (1994) have shown that although the active cue is water soluble, its action is not reversible by rinsing treated seeds.

Some particularly recalcitrant seeds will only respond to smoke treatment after a period of seed aging or soil storage (Roche *et al.* 1997a, Keeley & Fotheringham 1998a, Tieu *et al.* 2001b). This appears to be related to interaction with the soil environment rather than aging *per se* (Keeley & Fotheringham 1998a).

Smoke treatment has stimulated the germination of many species previously difficult to germinate (e.g. Dixon *et al.* 1995), proving to be a useful tool in regeneration and horticultural work. Also of horticultural interest are Taylor & van Staden's (1996) findings that smoke extract can be used to stimulate root initiation and growth in hydrocotyl cuttings. This investigation was prompted by the observation in germination experiments of smoke's positive action on radicle emergence and lateral root development.

It should be noted that many of the species tested have failed to respond positively to smoke treatment (e.g. Brown 1993b, Dixon *et al.* 1995). A few species have also been found to have their germination inhibited by smoke. The South African Asteraceae *Helichrysum aureonitens* had only 10% of smoke treated seeds germinate compared to 34% in the control, with 82% viability (Afolayan *et al.* 1997). Negative reactions may be related to the level of smoke treatment applied, either through excessive time

of aerosol smoking (Dixon *et al.* 1995, Roche *et al.* 1997a) or concentration of smoke extract (Brown 1993b, Jager *et al.* 1996b). Brown *et al.* (1993) found that inhibition caused by excessive concentration of smoke extract could be reversed by leaching.

Smoke Application

These various experiments have used smoke derived from burning either a mixture of plant materials, or material from one species only. Baxter *et al.* (1995) compared the ability of smoke produced from a range of 27 montane grassland species to promote the germination of the test species, *Themeda triandra*. One species failed to produce germination levels above that of the control, and results were varied amongst the other species. While these results confirm that the active component in smoke is widespread, they recommend caution in the choice of species for smoke production.

In a comparison of various smoking techniques, the application of aerosol smoke was found generally to be more successful than either application of smoked water or pre-imbibition in diluted smoked water (Roche *et al.* 1997b).

Smoke Cue in Soil

It also is not known what the quantity or duration of the active substance is in soil after a fire (Brown *et al.* 1993), though Baldwin *et al.* (1994) demonstrated that the smoke cue could remain active in soil for at least 53 days under greenhouse conditions.

Brown *et al.* (1994) suggest that smoke treatment is most effective on seeds stored on the soil surface, though the water solubility of the active compounds should allow its effects to reach seeds buried deeper in the soil profile. Dixon *et al.* (1995) hypothesise that adsorption of ammonia (a major inorganic component of smoke eluates) onto soil and subsequent leaching may allow movement of the active ingredients through the substrate. Interestingly, Roche *et al.* (1997b) found that six seed bank species showed improved germination when other pre-smoked seeds were buried with them, indicating that residual active smoke ingredient moved from the treated seeds into the surrounding soil.

It must be noted that the combustion of organic matter within the soil profile also provides a smoke effect. Heated soil has been shown to have a similar effect to smoke or charred wood treatment on some smoke-stimulated species (Keeley & Nitzberg 1984, Blank & Young 1998). While in the natural environment this would provide for another method of smoke delivery to seeds during a fire, it complicates experiments that involve the heating of soil to provide a heat-shock stimulus to buried seeds. This can not be considered to be a pure heat-shock treatment due to the confounding effect of the potential smoke release.

Multiple Germination Cues

Multiple germination cues in general are not uncommon (Bradbeer 1988). However, the action of multiple fire-related cues is yet to be examined in detail.

It has been suggested that heat and smoke are complementary germination triggers, acting on different species within the soil seedbank (Read *et al.* 2000, Enright & Kintrup 2001). However, some species have been shown to respond to both heat and smoke, or both heat and charred wood. Different interactions between the two cues have been seen in different species: equal (*Rhus trilobata*, California; Keeley 1987); equal and additive (*Eriodictyon crassifolium*, California; Keeley 1987; *Epacris stuartii*, Tasmania; Keith 1997); unequal and additive (various *Grevillea* species, Sydney; Morris 2000); and unequal and synergistic (*Epacris tasmanica*, Tasmania; Gilmour *et al.* 2000; *Phacelia cicutaria*, California; Keeley *et al.* 1985).

Conclusion

The post-fire environment is an advantageous time for seedling establishment, and most species in fire-prone habitats only recruit new seedlings at this time. Thus, establishment needs to be linked to the passage of fire. For species with soil-stored seedbanks, fire has several mechanisms with which to trigger seed germination. Physical dormancy is broken by heat and possibly by smoke, while physiological dormancy may be alleviated by chemicals released from smoke and charred wood. In seeds possessing both seed coat and embryo dormancy methods, multiple cues may be required for optimal germination to occur. The effects of combinations of the different fire-related germination cues have been studied systematically in very few species, and the ecological consequences of responding to multiple fire-related cues has not been investigated.

Thesis Aims

Approximately 89% of species in fire-prone vegetation types of the Sydney region are assumed to have a soil seedbank. While a post-fire germination pulse is common for such species, the mechanisms involved in fire-related dormancy breaking are known for very few species in the region other than legumes.

The effects of the fire-related germination cues provided by soil heating and combustion products (smoke and charred wood) have been studied on numerous plant taxa in several regions of the world. However, these different cues have rarely been studied in combination.

The general aim of this thesis was to investigate the effect of fire-related germination cues on a variety of soil seedbank species of the Sydney region. This involved exploring methods of laboratory application of three fire-related cues (heat, smoke, and charred wood); assessing the individual and interactive effects of these cues on germination response (dormancy breaking) in laboratory, glasshouse and field trials; and examination of how these cues are received by soil-stored seeds.

Overview of Experimental Aims

- Smoke and charred wood application (Chapter 3)
    - Find the best method of production of smoke and charred wood
    - Find the best method and concentration of smoke and charred wood application
- Heat range (Chapter 4)
    - Find the best temperature for heat-shock treatment
    - Compare heat sensitivity of different seed types
- Multiple germination cues (Chapter 5)
    - Investigate the individual and interactive effects of smoke, charred wood, and heat
    - Relate germination response to species' traits
- Germination cue effects on buried seeds (Chapter 6)
    - Investigate factors influencing smoke movement through soil
    - Application of germination cues to buried seeds in the field, and comparison with the effects of a fire
- Germination cue effects on a natural soil seedbank (Chapter 7)
    - Application of germination cues to a natural seedbank under glasshouse conditions
    - Application of germination cues to a natural seedbank in the field, and comparison with the effects of a fire

CHAPTER 2: SITE AND SPECIES DETAILS

Study Area

All species studied are natives of the Sydney region (NSW, Australia; Sydney latitude 33° 50' S, longitude 151° 15' E), occurring in the fire-prone vegetation communities of the Hawkesbury Sandstone. Sydney has a warm temperate climate with an average annual rainfall of 1222 mm. Winter temperatures average 8.7 (minimum) to 16.9 °C (maximum), and summer temperatures average 17.0 to 24.4 °C (Bureau of Meteorology climate averages data).

All seed collection was from Ku-ring-gai Chase National Park in Sydney's north. Field experiments were performed in Ku-ring-gai Chase and Heathcote National Parks, and soil for glasshouse seedbank experiments was collected from Ku-ring-gai Chase. See Map 2.1 for a map of the Sydney region showing these parks, and Map 2.2 for detail of Ku-ring-gai Chase National Park.

Ku-ring-gai Chase National Park is situated on the Hornsby Plateau, with a topography of sandstone slopes and plateaus with large sandstone outcrops (Chapman & Murphy 1989). The shallow, infertile soils support dry sclerophyll shrubland, woodland and open forest (Benson & Howell 1994). The habitats studied fall under the classification of Sydney Sandstone Ridgetop Woodland complex (map unit 10ar of Benson & Howell 1994), which includes woodland, open-woodland, low woodland and open-scrub. Outcrops of Coastal Sandstone Heath (map unit 21g) occur within the Ridgetop Woodland, and some of these communities (open-heath/closed scrub and rocky outcrop heath) have also been utilised. The woodland communities (characteristic tree species: *Eucalyptus gummifera* and *Eucalyptus haemastoma*) have a rich understorey of sclerophyllous shrubs which is floristically similar to the scrub and heath communities. All communities are dominated by species from the families Proteaceae, Myrtaceae, Fabaceae, and Epacridaceae. The structure and floristics of these broad communities varies locally with aspect, soil, drainage, and fire history (Benson & Howell 1994).

Ku-ring-gai Chase National Park is located in a highly fire-prone area, typified by steep slopes, flammable vegetation and rapid fuel accumulation. Large wildfire events have occurred in the Park approximately twice per decade in the period since records have been kept (since 1943), usually in summer and coinciding with extended drought periods. Prescribed burning is carried out regularly in autumn and winter by the NSW National Parks and Wildlife Service for hazard reduction purposes, contributing 18% of the total cumulative area burnt (Conroy 1996).
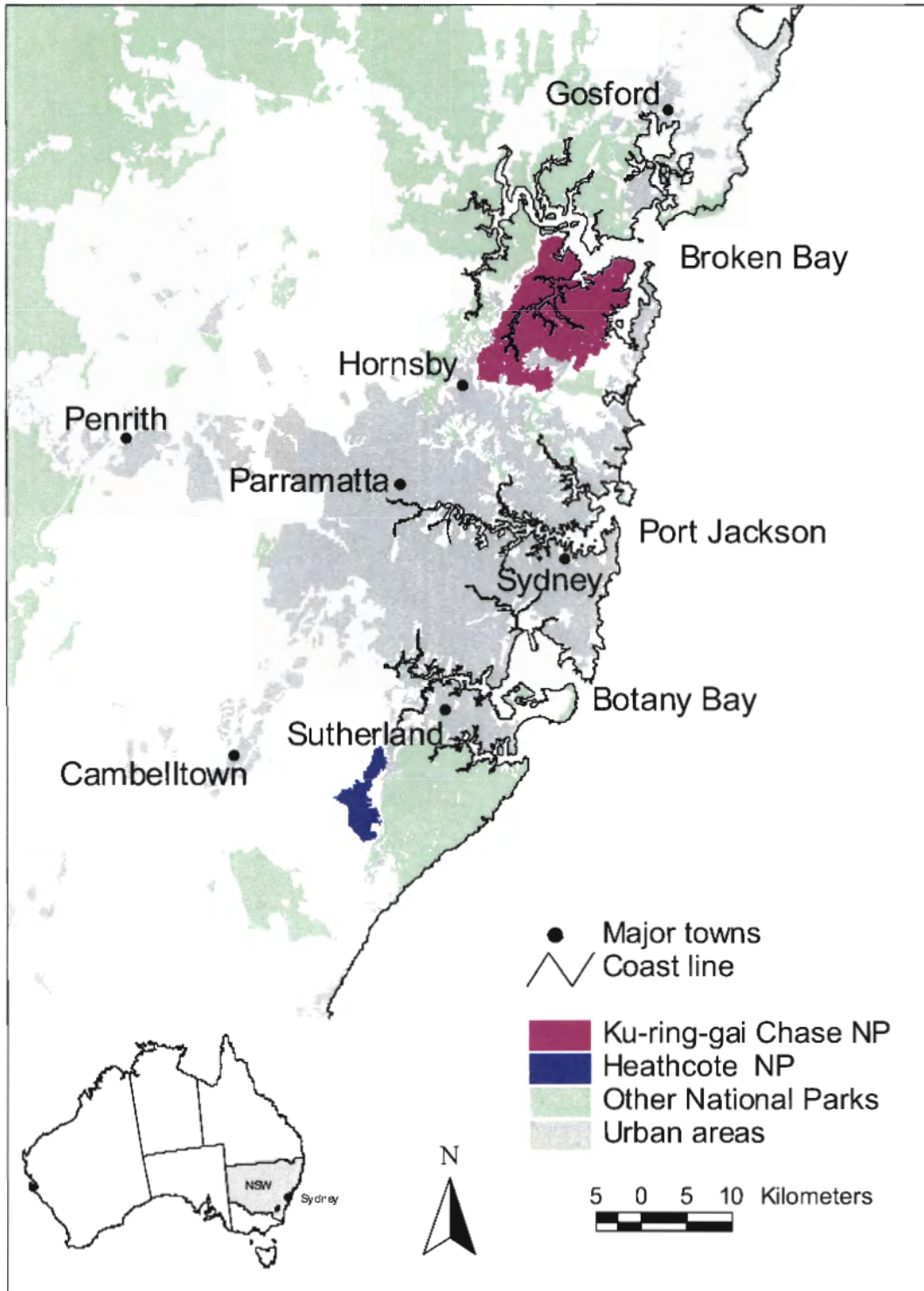
Map 2.1 Map of Sydney region, Australia. Areas used for seed collection (Ku-ring-gai Chase National Park), soil collection (Ku-ring-gai Chase National Park), and field experiments (Ku-ring-gai Chase and Heathcote National Parks) are shown.

Map 2.2 Map of Ku-ring-gai Chase National Park, from Sydney 1:100K map sheet; Park boundary outlined. Sites shown: sites 1-15, seed collection (see Table 2.2); site 15, soil collection; site 16, Myall Track prescribed burn, soil collection and field plots.

Species Details

All species studied here release their seeds upon maturity to form a soil-stored seedbank. A wide range of species has been chosen, to represent various seed morphologies, degree of seedbank persistence, and other traits (e.g. fire response) that influence population persistence through fire regimes.

Plant traits that will influence the way in which a population persists through fire events and regimes were examined for the study species. Traits of interest were: fire response (obligate seeder, resprouter, or variable), seedbank (persistent or transient soil storage), post-fire flowering (exclusive or facultative pyrogenic flowering), seed dispersal (wide or local) and seedling establishment (intolerant or tolerant) (Table 2.1). From combinations of these traits, species were categorised into four functional groups reflecting their dependence on post-fire seedling recruitment. Group 1 (post-fire recruitment essential) contains obligate seeders with persistent seedbank, local dispersal and intolerant establishment (equivalent to GI and SI species types of Noble & Slatyer 1980; see Tables 1.4 & 1.5). Group 2 (seedling recruitment important but not critical after every fire) contains obligate seeders with wide dispersal (DI) and species with a variable fire response, persistent seedbank, local dispersal and intolerant establishment. Group 3 (seedling recruitment not essential) contains resprouters with persistent seedbank, local dispersal, and intolerant establishment (VI); as well as resprouter or variable fire response species with persistent seedbank and any of the traits of post-fire flowering (Σ), wide seed dispersal (Δ), or tolerant establishment (VT). Group 4 is the species that would not normally encounter a fire-related germination cue, resprouters with transient seedbanks and rapid post-fire flowering (U).

The trait information was obtained from the NSW Flora Fire Response Database collated by the NSW National Parks and Wildlife Service from a wide range of data sources (Kenny & Bradstock 2001). For many species, both seeder and resprouter responses have been recorded by different observers, although there is usually a majority of recordings for one response. The categories of "seeder with limited resprouting capacity" (here termed facultative seeders) and "resprouter partly fire-sensitive" are classed here as species with a variable fire response.

Fruit type and possession of a hard seed coat has also been listed (Table 2.1). Species nomenclature follows Harden (1990-1993) except where recent name changes have been accepted by the NSW Herbarium.

Table 2.1 Taxonomic and trait details of the study species; * not identified to subspecies or variety. Life form (LF) categories: C = climber, H = herb, S = shrub. Fire response (FR): S = obligate seeder, S r = variable fire response (facultative seeder), R = resprouter, R s = variable fire response. Seedbank (SB): P = persistent, T = transient. Establishment: I = intolerant, T = tolerant. Seed dispersal: a- = adapted for. Functional group (FG): 1 = post-fire seedling recruitment essential, 2 = recruitment important but not critical, 3 = recruitment not essential, 4 = species without persistent seedbanks (see text for full details).

Species	Family	LF	FR	SB	Est.	Pyrogenic flowering	Seed dispersal mechanism	Seed dispersal distance	Fruit type	Hard seed coat?	FG
Monocotyledons											
Burchardia umbellata R.Br.	Colchicaceae	H	R	T		facultative		local	capsule		4
Dianella caerulea Sims *	Phormiaceae	H	R	T			vertebrate	wide	berry	yes	4
Dianella revoluta R.Br. *	Phormiaceae	H	R	T?		yes	a-vertebrate	wide	berry	yes	4
Doryanthes excelsa Corr.	Doryanthaceae	H	R	T		yes		local	capsule		4
Gahnia sieberiana Kunth	Cyperaceae	H	R	P	I	facultative	wide	wide	nut		3
Haemodorum planifolium R.Br.	Haemodoraceae	H	R	T		facultative		local	capsule		4
Lomandra longifolia Labill.	Lomandraceae	H	R	P	T	facultative	a-ant	local	capsule		3
Patersonia glabrata R.Br.	Iridaceae	H	R	P		facultative	a-ant	local	capsule		3
Thelionema caespitosum (R.Br.) R.J.F.Hend.	Phormiaceae	H	R	T?				local	capsule	yes	3
Xanthorrhoea resinifera (Sol. ex Kite) E.C.Nelson & D.J.Bedford	Xanthorrhoeaceae	H	R	T	I	exclusive		local	capsule		4
Dicotyledons											
Acacia linifolia (Vent.) Willd.	Fabaceae: Mimosoideae	S	S	P			ant	local	pod	yes	1
Acacia oxycedrus Sieber ex DC.	Fabaceae: Mimosoideae	S	S r	P	I		ant	local	pod	yes	2
Acacia suaveolens (Sm.) Willd.	Fabaceae: Mimosoideae	S	S	P	I		ant	local	pod	yes	1
Acacia terminalis (Salisb.) Macbride	Fabaceae: Mimosoideae	S	S r	P	I		ant	local	pod	yes	2
Actinotus helianthi Labill.	Apiaceae	H	S	P	I		wind	local	mericarp		1
Actinotus minor (Sm.) DC.	Apiaceae	H	S r	P	I		a-ant	local	mericarp		2
Astrotricha floccosa DC.	Araliaceae	S	S?	P?	I			local	schizocarp		1
Baeckea imbricata (Gaertn.) Druce	Myrtaceae	S	R	P	I	yes		local	capsule		3
Bauera rubioides Andrews	Baueraceae	S	R s	P	I		a-ant	local	capsule		2
Boronia ledifolia (Vent.) J.Gay	Rutaceae	S	S	P	I		a-ant	local	follicle	yes	1
Calytrix tetragona Labill.	Myrtaceae	S	R s	P	I		wind	local	indehiscent		2
Cassytha pubescens R.Br.	Lauraceae	C	S	P	I		a-vertebrate	wide	drupe		2
Conospermum taxifolium Sm.	Proteaceae	S	R s	P	I			local	nut		2
Dillwynia retorta (Wendl.) Druce	Fabaceae: Faboideae	S	S	P	I		ant	local	pod	yes	1
Dodonaea triquetra Wendll.	Sapindaceae	S	S	P	I		a-ant	local	capsule	yes	1
Epacris microphylla R.Br. var. *microphylla*	Epacridaceae	S	S r	P	I			local	capsule		2

Species	Family	LF	FR	SB	Est.	Pyrogenic flowering	Seed dispersal mechanism	Seed dispersal distance	Fruit type	Hard seed coat?	FG
Eriostemon australasius Pers.	Rutaceae	S	S r	P	I		a-ant	local	follicle	yes	2
Grevillea buxifolia (Sm.) R.Br. subsp. buxifolia	Proteaceae	S	S r	P	I		ant	local	follicle		2
Grevillea sericea (Sm.) R.Br.	Proteaceae	S	S r	P	I		a-ant	local	follicle		2
Grevillea speciosa (Knight) McGillivray	Proteaceae	S	S r	P	I		a-ant	local	follicle		2
Hibbertia scandens (Willd.) Gilg	Dilleniaceae	C	R s	P	I		bird	wide	follicle		3
Kunzea ambigua (Sm.) Druce	Myrtaceae	S	S	P	I			local	capsule		1
Kunzea capitata Rchb.	Myrtaceae	S	S r	P	I		ant	local	capsule		2
Lasiopetalum ferrugineum R.Br. subsp. ferrugineum	Sterculiaceae	S	R s	P	I		a-ant	local	capsule	yes	2
Mitrasacme polymorpha R.Br.	Loganiaceae	H	R s	P	I	facultative		local	capsule		3
Ozothamnus diosmifolius (Vent.) DC.	Asteraceae	S	S r	P			a-wind	local	achene		2
Persoonia pinifolia R.Br.	Proteaceae	S	S	P	I		vertebrate	wide	drupe		2
Phebalium squamulosum Vent. subsp. squamulosum	Rutaceae	S	R s	P	I		a-ant	local	follicle	yes	2
Pimelea linifolia Sm. subsp. linifolia	Thymelaeaceae	S	S r	P	I		a-ant	local	nut		2
Sprengelia incarnata Sm.	Epacridaceae	S	S	P	I			local	capsule		1
Telopea speciosissima (Sm.) R.Br.	Proteaceae	S	R	T		yes	wind	local	follicle		4
Woollsia pungens (Cav.) F.Muell.	Epacridaceae	S	S r	P	I			local	capsule		2
Zieria laevigata Bompl.	Rutaceae	S	S	P	I			local	follicle	yes	1

Seed Details

Seed Collection

Choice of species was determined largely by seed availability, i.e. the ability to find sufficiently large populations of mature plants to collect adequate quantity of seed without depleting the seedbank to the detriment of the population. Once suitable populations were located they were monitored for flowering and fruit development until mature seeds could be collected.

Seeds were either collected from within Ku-ring-gai Chase National Park between May 1997 and December 1999, or purchased from Harvest Seeds (Terrey Hills, NSW) in July 1999. For each species seed collection was performed at one site only, unless additional sites were required for sufficient quantity of seed (*Boronia ledifolia, Grevillea buxifolia,* and *Persoonia pinifolia*). Seeds were collected at maturity by either removal of the fruit from the plant, collection of fruits from the ground (*Persoonia pinifolia*), or covering fruit bearing branches with muslin bags to allow for explosive seed dispersal (Rutaceae species).

For some species multiple collections were made at different times (season or year). For these species seed lots have been defined to identify this, and separate germination trials were run on individual seed lots. Where seed quantity was low, seed lots were combined for some germination trials (*Grevillea buxifolia* and *Woollsia pungens*). Collection date, collection site, and seed lot definition are given in Table 2.2; site locations specified in Table 2.2 are shown on Map 2.2.

Seed Size

Average seed size is given in Table 2.3. For most species (those with a length >1 mm) seed mass and size was measured from 10 individual seeds. As seeds are not of a regular shape, measurements were taken at the largest point of each dimension (length, width and depth). For seeds with a round shape, only length and diameter were measured. No measurements were made of *Haemodorum planifolium* seeds before germination trials were performed, and no seed remained after trials.

Seed variance gives an indication of seed shape. This is calculated as the variance of seed length, width and depth after transforming all values so that length is unity. Variance has a minimum value of zero in perfectly spherical seeds, and a maximum of 0.3 in needle- or disc-shaped seeds (Thompson *et al.* 1993).

Seed mass for small seeds (<1 mm) is given as the average number of seeds per mg. These figures are averages from several germination trials in which a quantity of seeds were weighed (between 0.005 and 0.02 g) and the total number of seeds counted. No size measurements were made for these seeds, and a visual assessment of seed shape was used to estimate seed variance.

Table 2.2 Seed collection details. Where more than one distinct collection time (season or year) was made for a species, seed lots are defined (a, b, c). P = seeds purchased. Site locations are shown in Map 2.2 (labelled by site number).

Species	Seed lot	Date collected	Collection site
Acacia linifolia		11/98	3. America Bay Track
Acacia oxycedrus		12/98	9. McCarr's Creek Rd - Coal & Candle Dr
Acacia suaveolens	a	12/97	10. Smiths Creek East Track
Acacia suaveolens	b	10/98	10. Smiths Creek East Track
Acacia terminalis		10/97	3. America Bay Track
Actinotus helianthi		12/97	9. McCarr's Creek Rd - Coal & Candle Dr
Actinotus minor		P 7/99	n/a
Astrotricha floccosa		11/97	1. West Head Rd near Resolute
Baeckea imbricata		3/99	8. McCarr's Creek Rd - Coal & Candle Dr
Bauera rubioides		12/98	4. West Head Rd north of Wilunga Track
Blandfordia grandiflora		P 7/99	n/a
Blandfordia nobilis		P 7/99	n/a
Boronia ledifolia		12/97	3. America Bay Track & 6. Centre Track
Burchardia umbellata		12/98	8. McCarr's Creek Rd - Coal & Candle Dr
Calytrix tetragona		P 7/99	n/a
Cassytha pubescens		11/98	5. Waratah Track
Conospermum taxifolium	a	11/97	8. McCarr's Creek Rd - Coal & Candle Dr
Conospermum taxifolium	b	11/99	8. McCarr's Creek Rd - Coal & Candle Dr
Dianella caerulea		P 7/99	n/a
Dianella revoluta		P 7/99	n/a
Dillwynia retorta	a	11/97	9. McCarr's Creek Rd - Coal & Candle Dr
Dillwynia retorta	b	11/98	5. Waratah Track
Dodonaea triquetra		12/97	15. Ku-ring-gai Chase Rd north of Chase Track
Doryanthes excelsa		P 7/99	n/a
Epacris microphylla	a	11/98	5. Waratah Track
Epacris microphylla	b	11/99	5. Waratah Track
Eriostemon australasius	a	12/97	15. Ku-ring-gai Chase Rd north of Chase Track
Eriostemon australasius	b	11/98	15. Ku-ring-gai Chase Rd north of Chase Track
Gahnia sieberiana		10/98	10. Smiths Creek East Track
Grevillea buxifolia	a	12/97	3. Amercia Bay Track & 5. Waratah Track
Grevillea buxifolia	b	12/98	3. Amercia Bay Track & 5. Waratah Track
Grevillea sericea	a	9/97	3. America Bay Track
Grevillea sericea	b	1/98	3. America Bay Track
Grevillea sericea	c	1/99	3. America Bay Track
Grevillea speciosa	a	1/98	8. McCarr's Creek Rd - Coal & Candle Dr
Grevillea speciosa	b	2/99	8. McCarr's Creek Rd - Coal & Candle Dr
Grevillea speciosa	c	12/99	8. McCarr's Creek Rd - Coal & Candle Dr
Haemodorum planifolium		5/97	11. Cottage Point Rd near Taber Trig
Ozothamnus diosmifolius		P 7/99	n/a
Hibbertia scandens		P 7/99	n/a
Kunzea ambigua		P 7/99	n/a
Kunzea capitata		12/98	9. McCarr's Creek Rd - Coal & Candle Dr
Lasiopetalum ferrugineum		11/97	14. Birrawanna Track
Lomandra longifolia		P 7/99	n/a
Mitrasacme polymorpha		12/98	9. McCarr's Creek Rd - Coal & Candle Dr
Patersonia glabrata		P 7/99	n/a
Persoonia pinifolia		8/97	12. Long Track & 13. Duffy's Track
Phebalium squamulosum		11/98	4. West Head Rd north of Wilunga Track
Pimelea linifolia	a	11/97	15. Ku-ring-gai Chase Rd north of Chase Track
Pimelea linifolia	b	10/98	7. West Head Rd near Duck hole
Sprengelia incarnata		11/98	3. America Bay Track
Thelionema caespitosum		P 7/99	n/a
Telopea speciosissima		P 7/99	n/a
Woollsia pungens	a	10/97	15. Ku-ring-gai Chase Rd north of Chase Track
Woollsia pungens	b	10/98	15. Ku-ring-gai Chase Rd north of Chase Track
Xanthorrhoea resinifera		P 7/99	n/a
Zieria laevigata		12/97	9. McCarr's Creek Rd - Coal & Candle Dr

Table 2.3 Seed size details. Size parameters are given as mean ± standard error (n = 10). Weight of small (<1 mm length) seeds is given as mean number of seeds per mg, no dimensions measured. † = diameter given in width column for round seeds; nm = not measured (see text for details). Variance is calculated from the size measurements (see text); * variance has been visually estimated for seeds that were not measured.

Species	Weight (mg)	Length (mm)	Width (mm)	Depth (mm)	Variance
Acacia linifolia	28.8 ± 2.45	6.33 ± 0.17	3.35 ± 0.13	2.20 ± 0.082	0.114
Acacia oxycedrus	40.2 ± 2.70	5.13 ± 0.24	3.18 ± 0.080	3.01 ± 0.043	0.052
Acacia suaveolens	35.7 ± 2.75	7.28 ± 0.17	3.55 ± 0.073	2.23 ± 0.068	0.130
Acacia terminalis	47.6 ± 2.92	6.36 ± 0.19	4.27 ± 0.094	2.10 ± 0.042	0.112
Actinotus helianthi	1.5 ± 0.17	4.26 ± 0.11	2.31 ± 0.21	0.61 ± 0.048	0.193
Actinotus minor	0.57 ± 0.12	2.93 ± 0.082	1.33 ± 0.056	0.59 ± 0.028	0.168
Astrotricha floccosa	2.6 ± 0.27	2.76 ± 0.097	1.54 ± 0.048	1.21 ± 0.035	0.088
Baeckea imbricata	28.2 seeds/mg	nm	nm	nm	0.05*
Bauera rubioides	0.32 ± 0.07	1.76 ± 0.048	0.83 ± 0.052	†	0.095
Boronia ledifolia	7.5 ± 0.43	4.00 ± 0.021	2.08 ± 0.025	1.55 ± 0.022	0.104
Burchardia umbellata	1.7 ± 0.19	2.76 ± 0.13	1.97 ± 0.17	1.13 ± 0.096	0.099
Calytrix tetragona	0.8 ± 0.10	5.59 ± 0.35	0.58 ± 0.029	nm	0.266
Cassytha pubescens	35.3 ± 1.67	3.97 ± 0.040	3.60 ± 0.076	†	0.004
Conospermum taxifolium	1.8 ± 0.13	2.55 ± 0.050	2.43 ± 0.065	†	0.001
Dianella caerulea	5.5 ± 0.78	3.29 ± 0.084	2.51 ±0.097	1.87 ± 0.047	0.048
Dianella revoluta	4.1 ± 0.28	3.16 ± 0.078	2.12 ± 0.033	1.46 ± 0.037	0.074
Dillwynia retorta	6.4 ± 0.45	3.01 ± 0.12	2.22 ± 0.042	1.52 ± 0.055	0.063
Dodonaea triquetra	3.4 ± 0.18	2.41 ± 0.064	2.03 ± 0.056	1.44 ± 0.031	0.042
Doryanthes excelsa	88.6 ± 3.94	14.40 ± 0.50	10.67 ± 0.57	1.66 ± 0.15	0.211
Epacris microphylla	34.1 seeds/mg	nm	nm	nm	0.1*
Eriostemon australasius	21.1 ± 1.09	5.35 ± 0.13	2.85 ± 0.045	2.11 ± 0.050	0.101
Gahnia sieberiana	14.8 ± 1.00	4.66 ± 0.13	2.43 ± 0.037	2.46 ± 0.091	0.076
Grevillea buxifolia	58.6 ± 2.18	12.12 ± 0.26	3.62 ± 0.13	2.01 ± 0.046	0.201
Grevillea sericea	24.6 ± 0.91	9.16 ± 0.23	2.45 ± 0.28	1.37 ± 0.033	0.214
Grevillea speciosa	26.9 ± 0.68	9.65 ± 0.16	2.68 ± 0.063	1.27 ± 0.11	0.216
Haemodorum planifolium	nm	nm	nm	nm	0.2*
Hibbertia scandens	8.7 ± 0.62	3.40 ± 0.12	2.77 ± 0.090	1.86 ± 0.065	0.056
Kunzea ambigua	10.0 seeds/mg	nm	nm	nm	0.1*
Kunzea capitata	21.2 seeds/mg	nm	nm	nm	0.1*
Lasiopetalum ferrugineum	1.3 ± 0.10	2.07 ± 0.060	1.07 ± 0.015	†	0.077
Lomandra longifolia	12.0 ± 1.30	4.08 ± 0.15	2.57 ± 0.10	1.81 ± 0.066	0.081
Mitrasacme polymorpha	27.9 seeds/mg	nm	nm	nm	0.0*
Ozothamnus diosmifolius	16.1 seeds/mg	nm	nm	nm	0.15*
Patersonia glabrata	6.8 ± 0.35	5.63 ± 0.13	1.62 ± 0.044	1.45 ± 0.050	0.176
Persoonia pinifolia	221.7 ± 7.56	11.01 ± 0.17	6.20 ± 0.14	5.44 ± 0.093	0.076
Phebalium squamulosum	1.1 ± 0.09	2.41 ± 0.077	1.04 ± 0.022	0.80 ± 0.026	0.130
Pimelea linifolia	1.6 ± 0.15	3.10 ± 0.070	1.13 ± 0.030	0.96 ± 0.027	0.147
Sprengelia incarnata	33.4 seeds/mg	nm	nm	nm	0.0*
Telopea speciosissima	51.2 ± 3.89	7.55 ± 0.17	6.80 ± 0.20	2.41 ± 0.16	0.138
Thelionema caespitosum	1.6 ± 0.10	2.60 ± 0.030	1.54 ± 0.064	1.10 ± 0.026	0.090
Woollsia pungens	5.0 seeds/mg	nm	nm	nm	0.15*
Xanthorrhoea resinifera	8.7 ± 0.43	8.77 ± 0.24	3.68 ± 0.066	0.86 ± 0.045	0.209
Zieria laevigata	2.3 ± 0.20	3.08 ± 0.049	2.03 ± 0.063	1.20 ± 0.030	0.095

Seed Viability

Seed viability was assessed via a cut test performed at the end of each germination trial. Each seed was cut open to expose the embryo, and graded as viable (healthy, plump, white embryo), dead or empty. Given the large quantity of seed and number of experiments performed, this method was considered logistically preferable to performing tetrazolium tests. In the event that a treatment caused seed mortality, viability was calculated on a sub-set of the unaffected replicates. Average seed viability of the study species is given in Table 2.4.

For seed lots in which viability was calculated from more than one germination trial (see Table 2.4), the change in viability over time of seed storage was analysed with a one-way analysis of variance (ANOVA). There was no significant loss of seed viability in 18 of the 22 species tested. Seed viability declined with seed age ($P < 0.001$) in *Grevillea sericea* (seed lot c), *Phebalium squamulosum*, *Woollsia pungens* (seed lot a), and *Zieria laevigata*.

Germination results from laboratory experiments (Chapters 3-6) are expressed where possible as germination as a percentage of viable seed. This method has been used in preference to germination as a percentage of total seed to account for variation caused by the presence of inviable seeds within replicate seed batches. Germination as a percentage of total seed has only been used in cases where no accurate indication of seed viability could be obtained. Where mortality of seeds occurred due to experimental treatment, mean viability from the control treatment or a separate viability test was used to calculate germination as a percentage of potentially viable seed.

Table 2.4 Seed viability of study species. Viability (mean ± standard error) is given for each instance that it was measured or calculated for all replicate seed batches of a germination trial. Note that viability was measured at the end of a germination trial, based on the number of seeds that germinated and a cut test assessment of the viability of remaining ungerminated seeds. Seed lot is defined in Table 2.2. Seed age at the time of testing is given in months (estimated for purchased seeds). For species in which multiple experiments were performed on a single seed lot at different times this gives an indication of variation in viability with storage.

Species	Seed lot	Seed age	Viability	Species	Seed lot	Seed age	Viability
Acacia linifolia		8	88.7 ± 3.1	*Kunzea ambigua*		>3	94.7 ± 0.6
Acacia oxycedrus		7	99.3 ± 0.5			>4	96.0 ± 0.5
Acacia suaveolens	a	2	96.3 ± 0.8			>11	99.1 ± 0.2
		19	95.8 ± 1.0	*Kunzea capitata*		1	96.1 ± 0.5
		28	96.7 ± 1.5			13	94.3 ± 0.6
Acacia suaveolens	b	10	96.8 ± 1.1			15	95.4 ± 0.5
Acacia terminalis		10	99.4 ± 0.3	*Lasiopetalum ferrugineum*		3	91.0 ± 1.5
Bauera rubioides		2	93.1 ± 1.3			19	92.6 ± 1.3
		5	97.7 ± 0.4			20	96.3 ± 0.8
		30	92.9 ± 1.7	*Lomandra longifolia*		>1	70.5 ± 1.4
Boronia ledifolia		8	91.7 ± 1.8			>9	49.0 ± 3.6
		9	96.6 ± 0.7			>11	81.5 ± 1.7
		13	96.3 ± 1.5	*Mitrasacme polymorpha*		18	91.6 ± 0.9
Burchardia umbellata		1	97.5 ± 0.7	*Ozothamnus diosmifolius*		>3	84.6 ± 2.6
Calytrix tetragona		>9	81.8 ± 0.8			>4	81.1 ± 3.1
		>11	96.8 ± 0.7	*Patersonia glabrata*		>1	88.5 ± 1.4
Conospermum taxifolium	a	23	85.9 ± 1.3			>8	60.7 ± 3.5
		24	81.6 ± 1.4			>9	90.0 ± 3.5
		28	88.8 ± 1.9	*Phebalium squamulosum*		2	88.0 ± 2.9
Conospermum taxifolium	b	4	85.0 ± 1.0			3	93.1 ± 1.9
Dianella caerulea		>1	96.9 ± 0.8			7	96.6 ± 0.7
		>8	95.3 ± 1.2			19	82.3 ± 1.5
Dianella revoluta		>8	98.6 ± 0.6	*Pimelea linifolia*	a	31	88.1 ± 2.2
Dillwynia retorta	a	9	79.2 ± 1.9	*Pimelea linifolia*	b	10	81.4 ± 1.2
Dillwynia retorta	b	8	82.8 ± 2.0			12	81.8 ± 2.1
		17	80.4 ± 3.0			20	81.4 ± 1.6
Dodonaea triquetra		2	97.1 ± 0.4	*Sprengelia incarnata*		17	c. 100
		3	97.9 ± 0.4	*Thelionema caespitosum*		>1	95.5 ± 1.2
		19	98.5 ± 0.5			>3	96.8 ± 1.2
Epacris microphylla	a	14	92.1 ± 1.7			>11	94.0 ± 1.2
		16	97.0 ± 0.4	*Woollsia pungens*	a	11	97.9 ± 0.3
Epacris microphylla	b	4	96.6 ± 0.5			15	80.1 ± 1.2
Eriostemon australasius	a	9	90.0 ± 0.2	*Woollsia pungens*	a	32 } 74.3 ± 1.7
Grevillea buxifolia	a	17 } 90.4 ± 1.4		b	20 }
	b	5 }	*Xanthorrhoea resinifera*		>9	86.7 ± 3.5
Grevillea sericea	a	11	71.9 ± 2.4	*Zieria laevigata*		10	78.6 ± 1.6
Grevillea sericea	b	8	99.4 ± 1.3			13	67.6 ± 2.0
Grevillea sericea	c	8	97.9 ± 0.3				
		19	91.2 ± 1.1				
Grevillea speciosa	a	8	98.5 ± 0.5				
Grevillea speciosa	b	8	96.6 ± 0.5				
Grevillea speciosa	c	9	97.0 ± 0.7				
Haemodorum planifolium		20	84.6 ± 2.2				
Hibbertia scandens		>1	49.8 ± 2.4				
		>3	65.6 ± 2.7				
		>8	76.0 ± 2.8				

CHAPTER 3: SMOKE AND CHARRED WOOD APPLICATION

Aim

The next three chapters examine the effects of three fire-related germination cues (smoke, charred wood, and heat) through laboratory germination trials on individual species. Very few individual species (as opposed to soil seedbank studies) in eastern Australia have been examined for smoke and charred wood effects on germination. In experiments elsewhere (California, South Africa, Western Australia), variation has been found in the effects of smoke and charred wood applied by different methods and at different concentrations.

This chapter presents a series of experiments designed to look at various aspects of both smoke and charred wood as seed germination cues, and to find the most appropriate methods of application of these potential cues for further experiments (Chapter 5). The smoke experiments explore the method of application (smoked water versus aerosol smoke), concentration (amount of smoke applied), and whether smoke has any impact on water-impermeable seed coats. The charred wood experiments explore the source material (wood from different species), method of charred wood production (collection of naturally charred material and laboratory production), method of application (powdered versus solution), and concentration (amount of charred wood applied).

Methods

Smoke Concentration

Nineteen species (Table 3.1) were tested for the effect of a range of aerosol smoke applications on germination. Smoke was produced by burning mixed litter material (leaves and sticks) in a beekeeper's burner (c. 10 l capacity). Litter was collected from the habitat of the majority of species, Hawkesbury sandstone woodland with *Eucalyptus* and *Banksia* species dominant (Ku-ring-gai Chase National Park). The smoke was blown through tubing (approximately 1 m in length, such that the smoke was cool at emergence) into a sealed plastic box (c. 36 l capacity) where the seeds were held in uncovered Petri dishes. Seeds were smoke treated for a range of times between 1 and 40 minutes, and compared to a control (no smoke application). Initial trials used the treatments: control, 1, 2.5, 5, 10, 15 minutes smoke. The upper limit of this range was altered for further trials. These used either: control, 0, 1, 2.5, 5, 10, 20, 40 minutes smoke; or control, 0, 1, 5, 10, 20, 40 minutes smoke, depending on the number of seeds available. The treatment range used for each species is given in Table 3.1.

Smoke Concentration: Effect on Hard Seed Coats

A range of aerosol smoke applications were tested on six species with hard (water-impermeable) seed coats (Table 3.2). Longer fumigation times were used than in the previous smoke concentration experiment: 15, 30, 60 and 120 minutes. For comparison there were also control (untreated seeds), heat (80 °C for 10 minutes) and scarification (the distal end of the seed was rubbed across sandpaper by hand) treatments. Scarification was not applied for species with a limited seed quantity. Heat and scarification were used as 'control' treatments in this case, as species with a hard seed coat require these forms of treatment to break the physical dormancy (Cavanagh 1980). The particular heat treatment used (80 °C for

10 minutes) was chosen based on the peak temperature reported for similar species (Auld & O'Connell 1991).

Smoke Application Method: Aerosol vs. Solution

Aerosol smoke application was compared to the use of aqueous smoke extract ('smoked water'). Smoked water was produced by funnelling smoke from the bee keeper's burner (material burnt as in the smoke concentration experiment) into a 1000 ml armed conical flask containing 500 ml of distilled (reverse osmosis) water. Air was removed from the flask with a vacuum suction unit, causing the smoke to bubble through the water in the flask (de Lange & Boucher 1990, Baxter *et al.* 1994). Two solutions were made by continuing this process for different time periods (30 and 60 minutes).

These smoked water solutions (0.5 ml) were applied to seeds of one species, *Epacris microphylla* (seed lot a, average of 37 seeds per replicate, seed age 13 months, viability $92.1 \pm 1.7\%$). This application was compared to a control (distilled water only) and aerosol smoke treatment (15 minute smoke application; watered with distilled water). To avoid immediately diluting the applied solutions, the Petri dishes were sealed with parafilm for two weeks following treatment to prevent evaporation, after which they were watered (with distilled water) as necessary.

Charred Wood Concentration and Application Method

Eight species (Table 3.3) were tested for the effect on germination of a range of charred wood concentrations and application type; ground charred wood (charate) versus charred wood solution. Charred wood was collected by scraping charred material from the trunks of four tree species (*Eucalyptus haemastoma, Eucalyptus eximia, Eucalyptus paniculata, Persoonia levis*) burnt in a prescribed fire seven weeks prior to collection.

Charred wood from the four species was mixed and ground finely. A charate solution was made by mixing 25 g of charate with 500 ml distilled (reverse osmosis) water. This solution was agitated thoroughly and left to stand for 24 hours, before being filtered through a 0.5 mm mesh. Dilutions were then made from this 50 g/l stock solution.

The treatment levels of charred wood used were: control (distilled water only), 0.001, 0.005, 0.01, 0.05 g charate, and 0.1, 1, 2.5, 5, 10, 50 g/l charate solution. For species with fewer seeds available, a reduced range of treatments was used (control, 0.001 g, 0.05 g charate, 1 g/l, 5 g/l, 50 g/l charate solution), as stated in Table 3.3. Charate was spread over the filter paper within each Petri dish and 0.5 ml distilled water added. Charate solution treatments received 0.5 ml solution per Petri dish. The Petri dishes were sealed with parafilm for two weeks following treatment to prevent evaporation, after which they were watered (with distilled water) as necessary.

Table 3.1 Species tested in the smoke concentration experiment. Seed lot is defined in Table 2.2. Smoke range applied: 1 = 0, 1, 2.5, 5, 10, 15 minutes; 2 = 0, 1, 2.5, 5, 10, 20, 40 minutes; 3 = 0, 1, 5, 10, 20, 40 minutes of aerosol smoke application. For seeds that were weighed, the average number of seeds (av.) per replicate is given. Seed age at the start of trials is given in months (approximated for purchased seeds). Seed viability is given as mean ± standard error for viability measured during these experiments; * mean viability from another trial was used (see text).

Species	Seed lot	Range used	Seeds per replicate	Seed age (months)	Seed viability
Boronia ledifolia		1	10	13	96.3 ± 1.45
Burchardia umbellata		1	25	1	97.5 ± 0.71
Conospermum taxifolium	a	3	25	24	81.6 ± 1.38
Dianella caerulea		2	25	≥1	96.9 ± 0.83
Eriostemon australasius	b	1	15	2	90.0 *
Haemodorum planifolium		1	25	20	84.6 ± 2.18
Hibbertia scandens		2	25	≥1	49.8 ± 2.40
Kunzea ambigua		3	0.01 g, av. 100	≥4	96.0 ± 0.50
Kunzea capitata		1	0.01 g, av. 190	1	96.1 ± 0.45
Lomandra longifolia		3	25	≥1	70.5 ± 1.37
Mitrasacme polymorpha		1	0.005 g, av. 144	1	91.6 *
Ozothamnus diosmifolius		1	25	≥4	81.1 ± 3.08
Patersonia glabrata		2	25	≥1	88.5 ± 1.40
Phebalium squamulosum		1	25	2	88.0 ± 2.91
Pimelea linifolia	b	2	25	10	81.4 ± 1.18
Sprengelia incarnata		1	0.01 g, av. 324	2	c. 100
Thelionema caespitosum		2	25	≥1	95.5 ± 1.18
Woollsia pungens	a	1	0.01 g, av. 43	15	80.1 ± 1.24
Zieria laevigata		1	25	13	67.6 ± 2.02

Table 3.2 Species tested in the smoke concentration (hard seed coats) experiment. Seed lot is defined in Table 2.2. Seed age at the start of trials is given in months. Seed viability is given as mean ± standard error.

Species	Seed lot	Seeds per replicate	Seed age (months)	Seed viability
Acacia linifolia		10	8	88.7 ± 3.07
Acacia oxycedrus		15	7	99.3 ± 0.46
Acacia suaveolens	a	25	19	95.8 ± 0.98
Dillwynia retorta	b	25	8	82.8 ± 2.01
Dodonaea triquetra		25	19	98.5 ± 0.47
Lasiopetalum ferrugineum		25	20	96.3 ± 0.80

Table 3.3 Species tested in the charred wood concentration and application experiment. Seed lot is defined in Table 2.2. Charred wood range applied: 1 = control, 0.001 g, 0.005 g, 0.01 g, 0.05 g, 0.1 g/l, 1 g/l, 2.5g/l, 5 g/l, 10 g/l, 50 g/l; 2 = control, 0.001 g, 0.05 g, 1 g/l, 5 g/l, 50 g/l. For seeds that were weighed, the average number of seeds (av.) per replicate is given. Seed age at the start of trials is given in months. Seed viability is given as mean ± standard error for viability measured during these experiments; * mean viability from another trial was used (see text).

Species	Seed lot	Range used	Seeds per replicate	Seed age (months)	Seed viability
Bauera rubioides		1	25	5	97.7 ± 0.43
Epacris microphylla	a	1	0.005 g, av. 158	6	95.8 *
Kunzea capitata		1	0.005 g, av. 118	5	96.1 *
Lasiopetalum ferrugineum		2	25	19	92.6 ± 1.31
Mitrasacme polymorpha		1	0.005 g, av. 149	6	91.6 *
Phebalium squamulosum		2	25	7	96.6 ± 0.72
Sprengelia incarnata		1	0.005 g, av. 200	6	c. 100
Woollsia pungens	b	2	0.005 g, av. 29	8	74.3 *

Charred Wood Source Material

Wood was collected from four common shrub and tree species of Hawkesbury sandstone vegetation: *Banksia serrata*, *Banksia ericifolia*, *Eucalyptus haemastoma* and *Leptospermum trinervium*. Charred wood was produced using the methods of Wicklow (1977). Stem segments (<1 cm diameter) were placed in a 30 cc crucible and heated over a bunsen burner flame until charred (approximately 9 minutes) then the lid was placed over the crucible to cease combustion. The charred stems were then finely ground in a mortar and pestle, and this material was used to produce solutions.

Solutions for all species were made immediately after charred wood production. For *Banksia serrata* an additional solution was made from charred wood produced 1 month earlier (stored in a sealed sample tube until used). Solutions were made by placing 1 g of charate in 100 ml distilled water. This was agitated thoroughly and left to stand for 60 hours before filtering through a 0.5 mm mesh. This stock 10 g/l solution was also diluted to give a 1 g/l solution.

A germination trial was then performed on seeds of one species, *Kunzea capitata* (average of 50 seeds per replicate, seed age 13 months, viability 94.3 ± 0.6%). Treatments applied were 1 g/l and 10 g/l charate solutions from these four species (1 month old and fresh charred wood for *Banksia serrata*), 1 g/l and 10 g/l charate solutions from the previous charred wood trial (field collected charred wood, species mixed), and a distilled water control. 0.5 ml of solution or water was applied to each Petri dish. The Petri dishes were sealed with parafilm for two weeks following treatment to prevent evaporation, after which they were watered (with distilled water) as necessary.

General

Each treatment was performed on four replicate seed batches. Each replicate seed batch consisted of either a known (usually 25) number or a weighed quantity of seeds. For those batches that were weighed (very small seeded species) the total number of viable seeds per batch was calculated at the end of the trial. Details of the number of seeds per replicate, the seed lot used (seed lots are defined in Table 2.2), and the age of seeds when tested are given in Tables 3.1-3.3. Replicate seed batches were treated independently (Morrison & Morris 2000) for aerosol smoke application (i.e. application repeated four times) and charred wood source material (i.e. four quantities of stem charred per species). Treatments for smoked water and charred wood concentration can not be considered as independent as only one stock solution was made (one stock solution per time period for smoked water; one original stock solution for charred wood, diluted to different concentrations) and applied to all seed batches. See Morrison & Morris (2000) for a detailed discussion of the issue of pseudoreplication in germination experiments.

Following treatment, seeds were placed in Petri dishes lined with Whatman No. 1 filter paper and watered with solutions as described for each experiment or distilled water. Dishes were re-watered (water only, no solutions were reapplied) as required to maintain moisture, and periodically checked for germination. Germination was determined as being when the radicle emerged, and germinated seeds were removed from the Petri dish. Trials ran until no further germination was recorded for at least one week. Seeds were kept under ambient laboratory conditions in a cabinet purpose-built to hold large quantities of Petri dishes as sufficient space and time in temperature-controlled growth cabinets was not available. A fan built in to the rear of the cabinet allowed for control of humidity, this was run from a timer that was set depending

on ambient conditions. Seeds were thus kept in the dark, but checking of germination was done in the light.

At the end of each trial, viability of the remaining seeds was assessed via a cut test. Each seed was cut open to expose the embryo, and graded as viable (healthy, plump, white embryo), dead or empty. Germination was expressed as a percent of the number of viable seeds available per replicate (germinated plus viable remaining seed). Mean measured viability is given in Tables 3.1-3.3. For some species the cut test was difficult to perform and/or inconclusive (*Epacris microphylla, Eriostemon australasius, Kunzea capitata, Mitrasacme polymorpha, Sprengelia incarnata, Woollsia pungens*). For these species a value for mean viability from another trial performed on the same seed lot at a similar test age was used to calculate germination as a percent of viable seed (see Table 2.4). These are shown in Tables 3.1 & 3.3 as mean viability values only (no standard error given).

Treatment effects were assessed by one-way analysis of variance (ANOVA) and post hoc Tukey honestly significant difference (HSD) multiple comparison, after checking for homogeneity of variance via Cochran's test. Note that the assumption of independence of the replicates has been violated for the smoked water and charred wood concentration experiments.

Results

Smoke Concentration

No germination was recorded for four species (*Boronia ledifolia, Hibbertia scandens, Ozothamnus diosmifolius,* and *Thelionema caespitosum*). Ten species showed no significant effect ($P > 0.05$) of any level of smoke treatment (Table 3.4). Dormancy of most of these seeds was high: six species showed very low germination levels (<5%); two species had moderate germination (10-40%); two species had high levels of germination (>70%).

Five species showed improved germination ($P < 0.05$) with smoke (Table 3.4). Among these species there was no difference (Tukey HSD) between different smoke concentrations for *Kunzea ambigua* and *Kunzea capitata*. *Patersonia glabrata, Pimelea linifolia* and *Sprengelia incarnata* showed some variation in germination level with smoke concentration (Fig. 3.1), with a general increase in germination seen with increasing smoke concentration. The optimal smoke treatment for these three species was 20, 40, and 15 minutes of smoke respectively.

Smoke Concentration: Effect on Hard Seed Coats

No level of smoke tested had a significant effect on the germination of any of the test species ($P > 0.05$); hence a pooled mean for smoke is presented in Table 3.5. Heat (and scarification where tested) significantly increased the germination of all species ($P < 0.001$) except *Acacia linifolia*, which had a high control germination and reduced germination after heat treatment. For this species, this temperature induced a lethal heat response (ANOVA of seed mortality: $P < 0.05$).

Smoke Application Method: Aerosol vs. Solution

For the test species, *Epacris microphylla*, smoked water had the same positive influence on germination as did aerosol smoke application ($P = 0.002$). There was no significant difference (Tukey HSD) between the different smoke applications (smoked water 30 and 60 minutes, and aerosol smoke 15 minutes; Fig. 3.2).

Charred Wood Concentration and Application Method

Charred wood collected from the field had no significant effect on the germination of four of the eight species tested ($P > 0.05$; Table 3.6). Germination for the other four species was reduced by both charate and charate solution (Table 3.6), with a greater negative effect apparent with increasing charred wood concentration (Fig. 3.3).

Charred Wood Source Material

Germination level of the test species, *Kunzea capitata*, was significantly increased above the control by freshly produced charred wood from all source species except *Leptospermum trinervium* and *Banksia serrata* (older charred wood). There was no significant difference between concentrations for any of the charate solutions. There was no significant variation between the different species of source material; however, *Eucalyptus haemastoma*, *Banksia ericifolia* and *B. serrata* (older charred wood) resulted in the highest levels of germination (Fig. 3.4). Germination level was lower than the control for the field-collected charate solutions. This difference was not seen statistically when all data was analysed together as the variance for the five source species was much greater than that of the control or field charred wood (Fig. 3.4). Due to concern that this difference in variation may have hidden potential patterns between the control and field charred wood, this was tested with a separate ANOVA. A significant difference ($P = 0.001$) was found between control and field charred wood, though not between the two concentrations of field charred wood (Tukey HSD).

Table 3.4 Results of the smoke concentration experiment. Percentage germination (mean ± standard error) and ANOVA results: P value given for species with a significant smoke effect; nsd = no significant difference ($P > 0.050$). Mean germination is pooled across all smoke treatment; see Fig. 3.1 for species with differences among smoke treatments.

Species	Control	Smoke	ANOVA results
Burchardia umbellata	88.1 ± 2.99	72.3 ± 4.72	nsd
Conospermum taxifolium	4.1 ± 2.65	2.7 ± 0.66	nsd
Dianella caerulea	2.2 ± 1.26	2.7 ± 0.23	nsd
Eriostemon australasius	0.0 ± 0.00	1.1 ± 0.61	nsd
Haemodorum planifolium	83.5 ± 5.27	84.0 ± 1.79	nsd
Kunzea ambigua	28.6 ± 1.77	43.1 ± 1.44	$P = 0.004$
Kunzea capitata	14.4 ± 2.40	60.3 ± 3.85	$P < 0.001$
Lomandra longifolia	29.7 ± 4.91	37.8 ± 2.49	nsd
Mitrasacme polymorpha	0.0 ± 0.00	1.0 ± 0.35	nsd
Patersonia glabrata	15.2 ± 5.01	22.9 ± 2.64	$P = 0.029$; Fig. 3.1a
Phebalium squamulosum	1.0 ± 1.00	1.4 ± 0.62	nsd
Pimelea linifolia	2.3 ± 1.34	8.9 ± 1.46	$P = 0.005$; Fig. 3.1b
Sprengelia incarnata	0.4 ± 0.15	9.9 ± 2.07	$P < 0.001$; Fig. 3.1c
Woollsia pungens	12.8 ± 4.54	12.0 ± 1.11	nsd
Zieria laevigata	3.8 ± 3.75	7.7 ± 1.49	nsd

Table 3.5 Results of the smoke concentration (hard seed coats) experiment. Percentage germination (mean ± standard error) and ANOVA results: P value given; different letters represent significant differences (Tukey HSD); n/a = not applicable (scarification treatment not performed).

Species	Control	Smoke	Heat	Scarification	ANOVA results
Acacia linifolia	51.8 ± 5.74 a	60.3 ± 4.87 a	12.5 ± 7.22 b	n/a	$P = 0.013$
Acacia oxycedrus	3.3 ± 1.93 a	2.1 ± 0.80 a	50.3 ± 5.20 b	n/a	$P < 0.001$
Acacia suaveolens	3.1 ± 1.99 a	3.9 ± 0.96 a	79.3 ± 2.42 b	100.0 ± 0.00 c	$P < 0.001$
Dillwynia retorta	3.4 ± 1.12 a	5.9 ± 0.97 a	92.4 ± 0.96 b	73.4 ± 4.35 b	$P < 0.001$
Dodonaea triquetra	4.0 ± 2.83 a	6.0 ± 0.81 a	94.0 ± 3.46 b	93.8 ± 2.70 b	$P < 0.001$
Lasiopetalum ferrugineum	3.3 ± 2.16 a	4.4 ± 1.22 a	89.8 ± 3.82 b	n/a	$P < 0.001$

Table 3.6 Results of the charred wood concentration experiment. Percentage germination (mean ± standard error) and ANOVA results: P value given for species with a significant charred wood effect; nsd = no significant difference ($P > 0.050$). Mean germination is pooled across all charred wood treatments; see Fig. 3.3 for species with differences among charred wood treatments.

Species	Control	Charred wood	ANOVA results
Bauera rubioides	35.9 ± 6.04	9.5 ± 1.28	$P < 0.001$; Fig. 3.3a
Epacris microphylla	36.7 ± 3.65	8.9 ± 1.23	$P < 0.001$; Fig. 3.3b
Kunzea capitata	84.9 ± 2.01	28.6 ± 4.85	$P < 0.001$; Fig. 3.3c
Lasiopetalum ferrugineum	10.8 ± 0.98	5.1 ± 1.11	nsd
Mitrasacme polymorpha	0.3 ± 0.20	0.2 ± 0.08	nsd
Phebalium squamulosum	21.7 ± 6.0	26.4 ± 1.9	nsd
Sprengelia incarnata	0.1 ± 0.13	0.1 ± 0.04	nsd
Woollsia pungens	20.3 ± 4.69	7.4 ± 5.19	$P = 0.002$; Fig. 3.3d
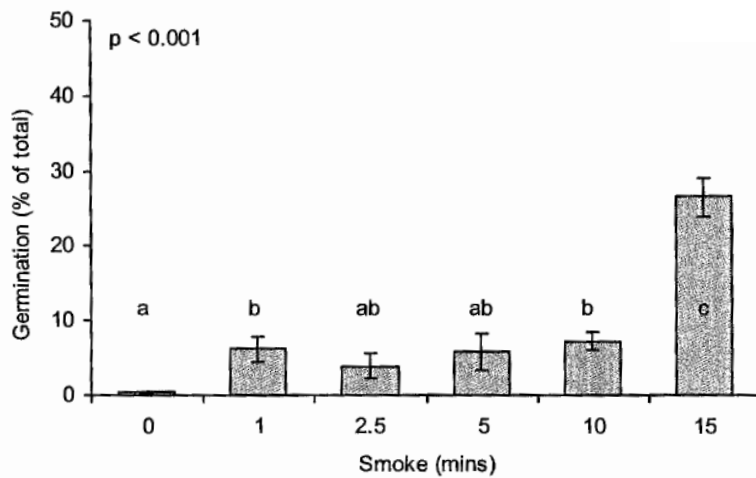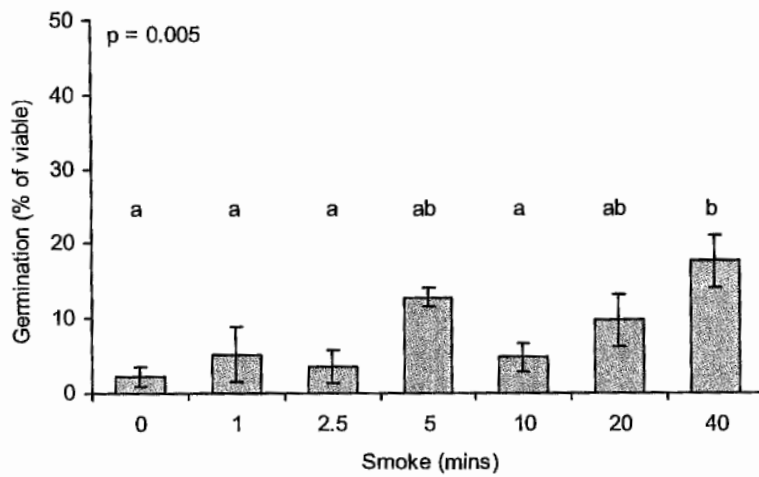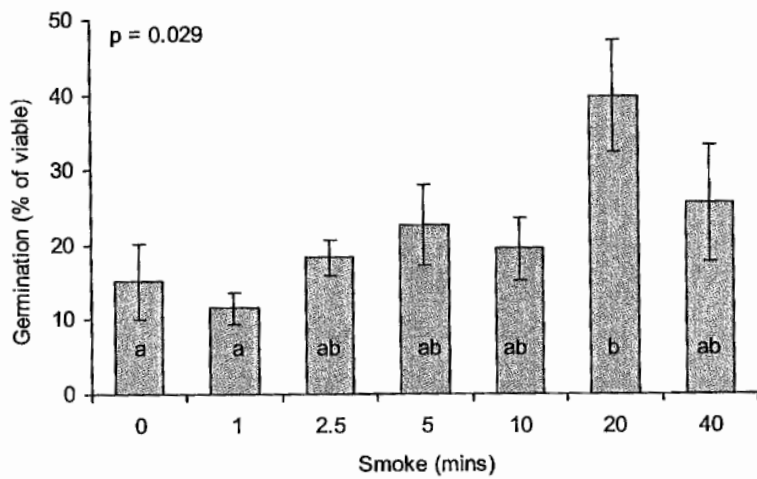
Figure 3.1 Germination responses of (a) *Patersonia glabrata*, (b) *Pimelea linifolia*, and (c) *Sprengelia incarnata* to a range of smoke concentrations. Error bars are standard error. ANOVA results: *P* value given; different letters represent a significant difference (Tukey HSD).
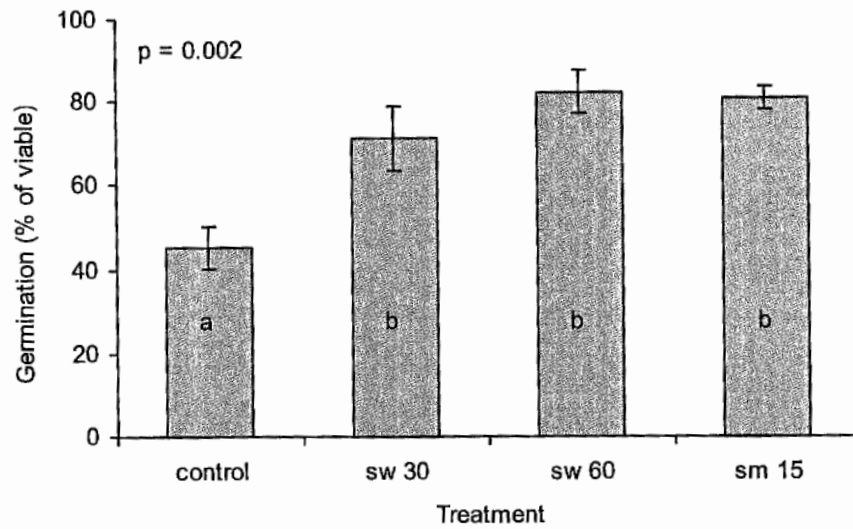
Figure 3.2 Germination response of *Epacris microphylla* to smoked water treatment. Treatments: sw 30 = smoked water (30 minute solution), sw 60 = smoked water (60 minute solution), sm 15 = aerosol smoke applied for 15 minutes. Error bars are standard error. ANOVA results: *P* value given; different letters represent a significant difference (Tukey HSD).
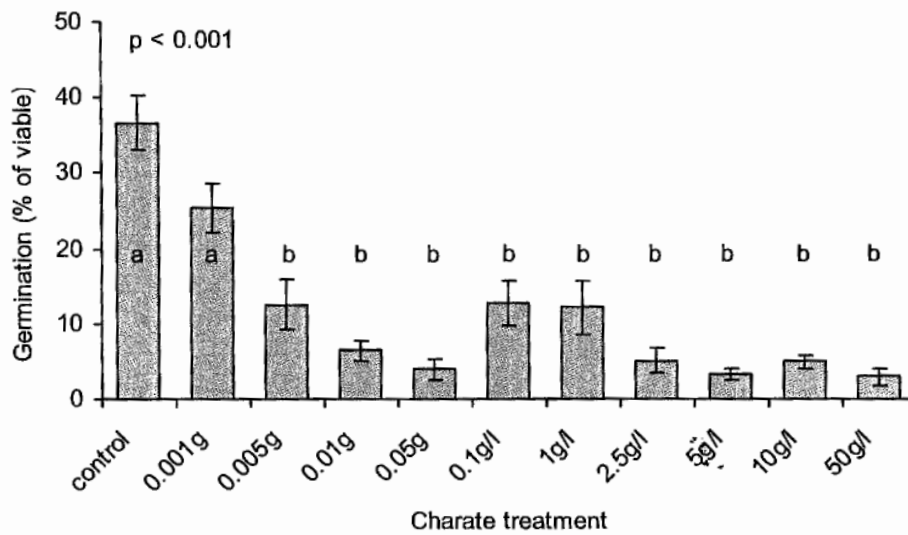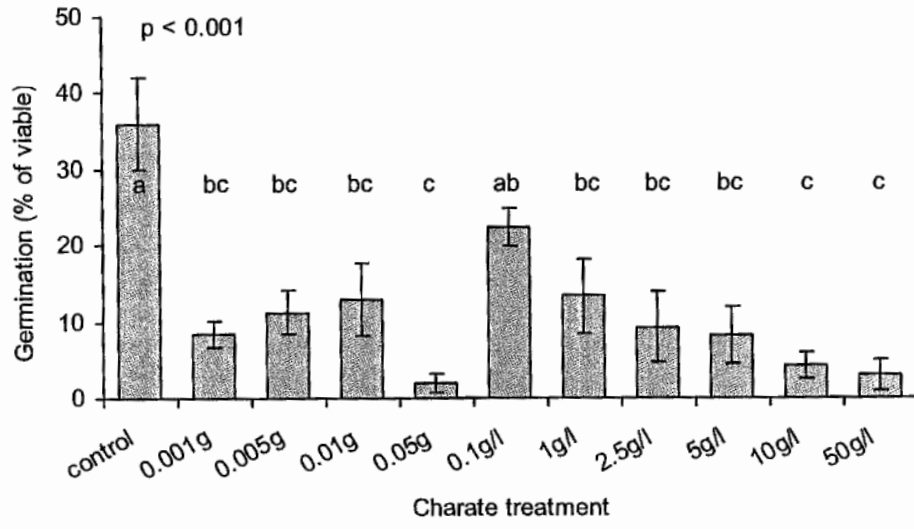
Figure 3.3 Germination responses of (a) *Bauera rubioides* and (b) *Epacris microphylla* to charred wood application method and concentration. Error bars are standard error. ANOVA results: *P* value given; different letters represent a significant difference (Tukey HSD).
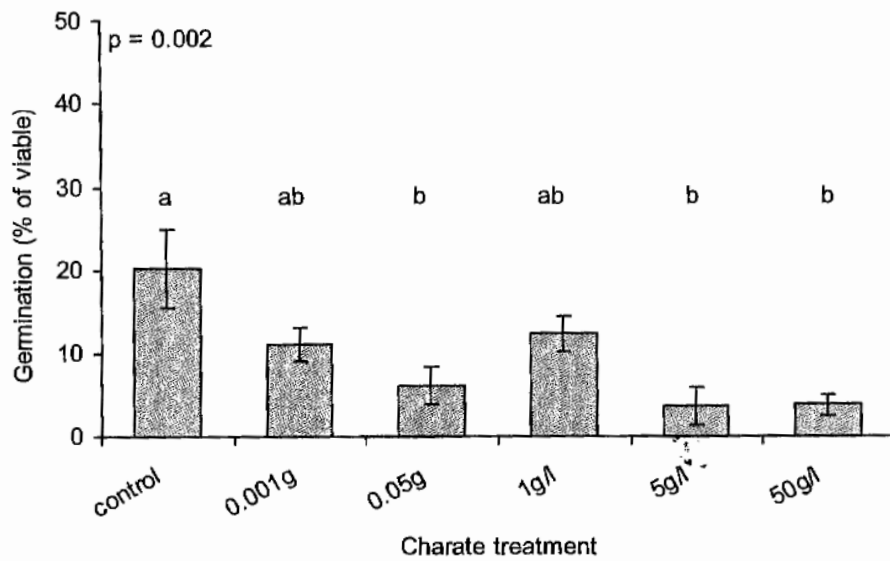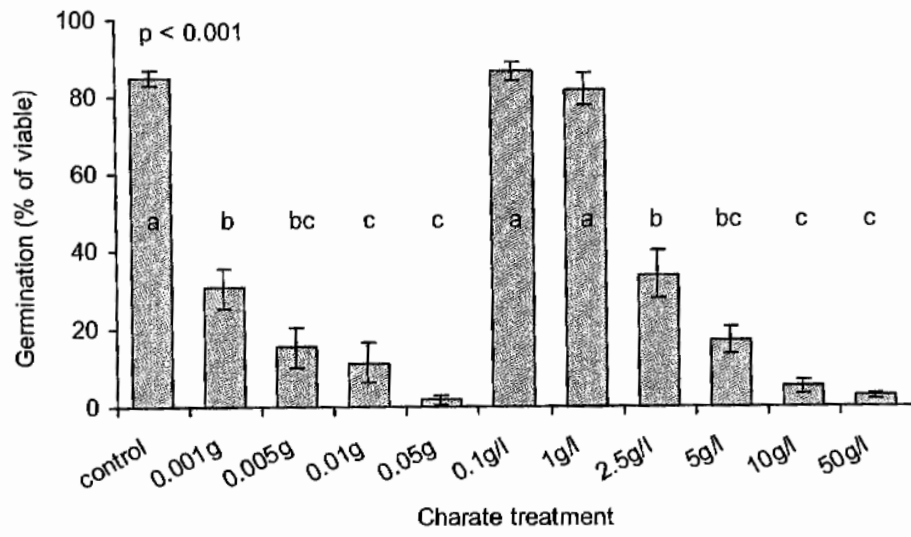
Figure 3.3 continued Germination responses of (c) *Kunzea capitata* and (d) *Woollsia pungens* to charred wood application method and concentration. Error bars are standard error. ANOVA results: *P* value given; different letters represent a significant difference (Tukey HSD).
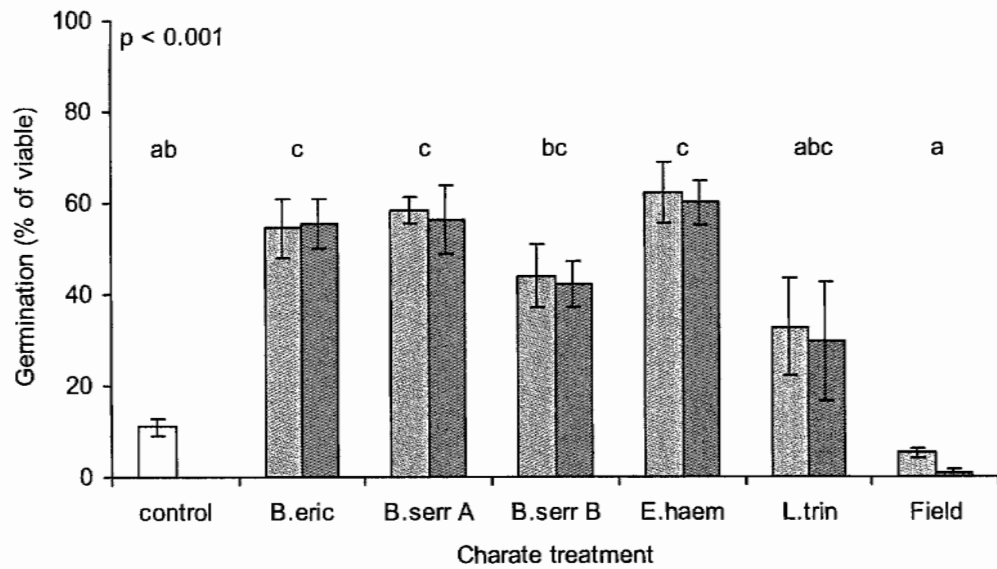
Figure 3.4 Germination response of *Kunzea capitata* to charred wood source and concentration. Charred wood source: B.eric = *Banksia ericifolia*; B.serr = *Banksia serrata* (A = one month old charate, B = fresh charate); E.haem = *Eucalyptus haemastoma*; L.trin = *Leptospermum trinervium*, Field = charred wood collected from burnt trees (charred wood concentration experiment). Charred wood concentration: white bars = control; light grey bars = 1 g/l charate solution; dark grey bars = 10 g/l charate solution. Error bars are standard error. ANOVA results: *P* value given; different letters represent a significant difference (Tukey HSD).

Discussion

Smoke

Since its discovery as a germination cue (de Lange & Boucher 1990) smoke has been shown to stimulate the germination of a large number of species across a wide range of families and plant forms, particularly in seeds considered previously very difficult to germinate (Brown & van Staden 1997). Given how prolific the response to smoke has been in similar floras (47% of species tested in fynbos; Brown & van Staden 1997; 48 and 54% of species tested in Western Australia; Dixon *et al.* 1995, Roche *et al.* 1997a respectively) it is perhaps surprising how few of the species tested here (26%) showed improved germination with smoke treatment.

One of the species showing no response here (*Burchardia umbellata*) has been shown elsewhere to have a positive response (Dixon *et al.* 1995, Roche *et al.* 1997a). However, seeds used here showed no dormancy (control germination 88%), while seed used by both Dixon and Roche had a moderate degree of dormancy (control germination 18 and 24% respectively).

For some of the other species not responding to smoke here, one or more Western Australian species within the genus have shown a positive smoke effect (Dixon *et al.* 1995, Roche *et al.* 1997a): *Conospermum* (two species), *Haemodorum* (one species), and *Mitrasacme* (one species). However, species within a genus do not always show similar responses: e.g. *Dianella* (four species positive smoke response, four species no significant smoke effect) and *Lomandra* (one species positive, six species no significant effect) (Roche *et al.* 1997a).

Where a positive smoke effect was seen, the effect of increasing the amount of smoke applied was either constant or a general increase in germination. Only *Sprengelia incarnata* showed a strong preference for a particular smoke level (15 minutes). Keeley & Fotheringham (1998a) also found no consistent pattern with a range of aerosol smoke applications over a range of species, while Baxter *et al.* (1994) found 15 minutes application to give highest germination levels.

The different application methods tested for smoke (aerosol and solution) were equally effective, supporting previous observations that the active principle in smoke is water soluble (de Lange & Boucher 1990, Keeley & Fotheringham 1998a). Roche *et al.* (1997b) found that under field conditions direct aerosol smoke application is more effective than smoked water (either as smoked water applied to the soil after sowing seeds or pre-imbibition of seeds in smoked water prior to sowing), possibly due to dilution through rainfall.

It has been suggested that smoke might have a scarifying effect, with seed coat changes seen following smoke treatment. Smoke has been shown to increase the permeability of the subdermal membrane in some water-permeable seeds, though without affecting the seeds imbibition (Egerton-Warburton 1998, Keeley & Fotheringham 1998a & b). While these species did not have hard seed coats, a few legume species have been reported with a smoke response (Roche *et al.* 1997a & b), prompting Roche *et al.* (1997b) to claim that the significance of heat in the germination of legumes should be re-evaluated. However, in one of these trials (Roche *et al.* 1997b) the legumes that responded to smoke were all encountered in natural soil seedbank plots treated with smoke, and most did not produce highly

significant results. Caution must always be taken when interpreting treatment effects from such studies, as it is not known how many seeds of the species were in each sample plot in the first place. In the other trial where smoke influenced legume germinability (Roche *et al.* 1997a), two of three tested *Hovea* species had significantly higher germination with smoke treatment, though these both had unusually high control germination (11 and 51%) for hard-seeded species. None of the hard seeded species tested here showed any germination response after smoke treatment, while all (except *Acacia linifolia*) were stimulated by the more typical germination cues for these species, heat and scarification. An atypical heat response (though not negative as seen here) has been shown previously for *A. linifolia* (Auld & O'Connell 1991). While Auld & O'Connell (1991) found some stimulation of germination of *A. linifolia* at 80-120 °C, the germination level was of a significantly lower magnitude than the other 34 Fabaceae species studied.

Charred Wood

In contrast to smoke, results from charred wood experiments in similar habitats have been very variable. While many chaparral species have shown a positive germination response to charred wood (Keeley *et al.* 1985, Keeley 1987), there has been little success in either the Mediterranean Basin (Keeley & Baer-Keeley 1999) or Australia (Bell *et al.* 1987, Marsden-Smedley *et al.* 1997, Enright & Kintrup 2001). In Australian studies, Bell *et al.* (1987) found only two out of forty jarrah understorey species tested with a significant positive charred wood enhancement; Marsden-Smedley *et al.* (1997) got a positive response to charred wood alone from only one exotic herb; and Enright & Kintrup (2001) found no charred wood effect on either seedling density or species richness. These latter two studies both found significant smoke effects for many species. Brown (1993a) was the first to find charred wood alone to positively influence the germination of a fynbos species, and believes that failures to illicit charred wood responses from fynbos species (e.g. Pierce & Moll 1994) may be related to the way in which the charate solutions were prepared. Charred wood has since been found to have a similar stimulatory effect to smoke on several fynbos species (Keeley & Bond 1997).

The two different charred wood experiments performed here produced very different results. The charred wood that was collected from the trunks of burnt trees was inhibitory to germination. However the charred wood produced in the laboratory by burning wood stimulated germination. Keeley & Nitzberg (1984) found great variation in results from a range of charate experiments, with both the medium on which seeds were placed and the moisture level of this medium confounding the charate effect. The differences seen were, however, more subtle than those seen here.

Ash has been found to be inhibitory to a wide range of species (Sweeney 1956, Thomas & Wein 1985, Thomas & Wein 1990, Neeman *et al.* 1993a & b, Gonzalez-Rabanal & Casal 1995, Facelli & Kerrigan 1996, Enright *et al.* 1997, Reyes & Casal 1998). Perhaps the charred wood collected from the field had undergone too high a level of combustion, resulting in similar inhibitory action as ash. Jager *et al.* (1996a) have demonstrated that volatilisation of the stimulatory compounds can occur at temperatures greater than 200 °C. Alternatively, the active components of the charred wood may have been lost (e.g. via leaching from rain) during the time lag from the production of this charred wood in the prescribed fire to its collection and use (7 weeks).

Both charate and charate solution had the same effect, with germination further reduced as the concentration of charred wood applied was increased. Similarly to smoke, the active ingredient in charred wood appears to be water soluble, with charate solution generally having the same effect as charate (Keeley & Nitzberg 1984, Keeley & Pizzorno 1986).

In the second charred wood experiment, charred wood had a positive effect on germination, but there was no concentration effect. There was some variation in the effectiveness of the different species used to make the charate, in contrast to the results of Keeley & Pizzorno (1986) who found charred wood effect to be independent of wood type.

Comparison of this positive charred wood effect (Fig. 3.4) to the negative effect in the previous experiment on the same species (Fig. 3.3c) shows a large difference in the control germination level. The second experiment was performed on older seed than the first, and it appears that the innate dormancy of the seed lot has increased during that time. However, since charred wood from this previous experiment was included as a treatment in the second, comparison of the charred wood effect is still possible. The charred wood effect remains the same across the experiments, with the field produced charred wood decreasing the germination below the control level.

Even within chaparral species, for which charred wood has been a very successful germination treatment, the same species have shown an equal or better response to smoke treatment (Keeley & Bond 1997, Keeley & Fotheringham 1998a). Some variation may be due to chemical differences in the material used: smoke is generally prepared by burning leaves, and charred wood from wood. Since smoke and charred wood appear to illicit the same physiological response within seeds (Keeley & Fotheringham 1998a), and smoke seems both more effective and less affected by variation in production and application method, it seems wise for future studies seeking fire-related germination responses to concentrate on smoke.

Outcome

From these experiments the application method and concentration of smoke and charred wood were set for the following experiments (Chapter 5). Smoke was applied as aerosol smoke fumigation of 15 minutes. Charred wood was produced fresh in the laboratory with *Eucalyptus haemastoma* as the wood source. Charred wood was produced either by the method of Wicklow (1977) as described in this chapter, or by heating in a muffle furnace. Charred wood was applied as either charate or 10 g/l solution, with the quantity applied varied depending on the size of Petri dish used (0.05 g or 1 ml in 9 mm dishes; 0.03 g or 0.7 ml in 5 mm (generally used for very small seeds) dishes).

CHAPTER 4: HEAT RANGE

Aim

The most obvious fire-related germination cue is the heat pulse resulting from soil heating. Seeds from various families (most notably leguminous species) possess a water-impermeable ('hard') seed coat, which responds very well to a heat-shock cue, as disruption of the seed coat allows imbibition and germination (Cavanagh 1987). Temperatures required for this kind of response have been shown to fall within the range 60-120 °C in species from a wide range of fire-prone habitats (Martin *et al*. 1975, Shea *et al*. 1979, Keeley *et al*. 1981, Trabaud & Oustric 1989, Auld and O'Connell 1991, Cocks & Stock 1997). This range of temperatures can be expected to occur in the top of the soil profile during a fire in the same variety of habitats (Beadle 1940, Floyd 1966, Bradstock *et al*. 1992, Bradstock & Auld 1995, Odion & Davis 2000, Smith *et al*. 2000). The actual level of heating encountered by buried seeds will vary spatially with factors such as the depth of seed burial, fire intensity and duration, litter cover, soil texture, and soil moisture at the time of the fire (Auld & O'Connell 1991, Atkins & Hobbs 1995, Bradstock & Auld 1995).

While the bulk of taxa within fire-prone communities have soil seedbanks (Auld *et al*. 2000), most studies of heat-shock as a germination cue have focussed on legumes and other species that possess an obvious water-impermeable seed coat. This chapter examines heat-shock as a dormancy-breaking cue for a wider variety of plant taxa. The species studied have been chosen to represent both traditional heat responders (hard-seeded species) and other species with a soil seedbank and a post-fire recruitment pulse (not hard-seeded). Additional species have been chosen to represent seeds which would not normally encounter heat (post-fire flowerers with transient seedbanks) to examine differences in heat sensitivity. Relationships between species' fire response and seed attributes and the influence of and sensitivity to heat are then examined.

As seeder species are reliant on seedling recruitment alone for population persistence after a fire, it is predicted that seeder species should exhibit greater post-fire seedling establishment than resprouter species (Keeley 1977). This could be achieved by higher levels of seed production, seed survival, or germination (Moreno & Oechel 1992). Barro & Poth (1988) and Moreno & Oechel (1991) have shown that seeds of seeder species are more tolerant of higher temperatures than those of resprouters, hypothesising that seeds of obligate seeders are better adapted to survive fire. For higher germination levels to occur it might be expected that seeders should have a stronger response to fire-related germination cues.

Methods

Germination Trials

Study species were tested for the effect of a range of temperatures on seed dormancy, germination, and mortality. Seed batches were placed for 10 minutes in an oven pre-heated to the specified temperatures. The temperature range used was either: control (ambient), 60, 70, 80, 90, 100 °C; or control, 60, 80, 100, 120 °C as stated in Table 4.1. Initial trials were done in the temperature range of 60-100 °C; later trials were done in the range 60-120 °C in order to further examine lethal temperature (intermediate

temperatures of 70 and 90 °C were excluded due to limited seed quantity). For each temperature, four replicate batches were treated independently per species (i.e. replicates heated at separate times; Morrison & Morris 2000).

Each replicate seed batch consisted of either a known or approximate number of seeds (usually 25) or a known weight of seeds. For those measured by weight (very small seeded species) the total number of viable seeds per batch was calculated at the end of the trial. Table 4.1 gives the number or weight and average number of seeds per replicate.

Once treated, each seed batch was placed in a separate Petri dish on filter paper (Whatman No. 1) kept moist with distilled (reverse osmosis) water and placed in the dark at ambient temperature. Germination was determined as being when the radicle emerged, and germinated seeds were removed from the Petri dish. Trials ran until no further germination was recorded for at least 1 week (trial period of 8-10 weeks, unless a longer time was required for commencement of germination).

At the end of a trial seed viability was assessed via a cut test. Each seed was cut open to expose the embryo, and graded as viable (healthy, plump, white embryo), dead, or empty. This assessment was used to calculate: mean seed viability, germination as a percentage of viable seed, and treatment-induced seed mortality.

For species where ungerminated seed suffered from fungal infection which affected seed viability (*Doryanthes excelsa, Haemodorum planifolium, Telopea speciosissima, Xanthorrhoea resinifera*), a separate cut test was performed on freshly imbibed seed. This was used for mean viability and to calculate germination as a percentage of viability for all replicates; seed mortality could not be analysed for these species. In species for which the cut test was difficult to perform and/or inconclusive (*Actinotus helianthi, Astrotricha floccosa, Epacris microphylla, Kunzea capitata, Mitrasacme polymorpha, Sprengelia incarnata*) germination is expressed as percentage of total seeds available, and seed mortality was not analysed. Where high temperature resulted in seed mortality, viability from the control and unaffected treatments were used as mean viability, and to calculate germination as a percentage of viability for adversely heat affected replicates. For all other species, mean viability is calculated over all treatments, germination was calculated as a percent of the viable seeds available in each replicate seed batch, and treatment-induced seed mortality was analysed. Mean viability is given in Table 4.1.

Where possible (see above), figures of germination are presented as the percentage of total seed that germinated, remained dormant, or died; other figures are presented as only the percentage of total seed that germinated.

Data Analysis
Percentage data were arcsine transformed to improve normality and variance homogeneity (Sokal & Rohlf 1987). Homogeneity of variance was then re-checked by Cochran's test. Germination and mortality data were analysed for treatment effects by a one-way analysis of variance (ANOVA) and post hoc Tukey honestly significant difference (HSD) multiple comparison for each species.

The results of the Tukey tests were used to define significant temperatures. Optimal temperature was defined as resulting in significantly increased germination compared to the control and/or other

treatments. Inhibitory temperature was defined as resulting in significantly decreased germination with respect to the control. Lethal temperature was defined as resulting in significant seed mortality (when mortality could be analysed statistically), or resulting in zero germination (and germination reduced below other treatments and/or control).

Relationships were sought between the effect of heat-shock treatment (germination response and seed mortality) and species and seed traits (functional group, seed coat, seed mass, and seed shape). Germination response to heat treatment was given three categories: no significant effect, negative heat effect, and positive heat effect. Seed mortality was grouped into species that showed a lethal temperature (as described above) and those that didn't. Seed coat type was divided into hard (i.e. water-impermeable) and soft (i.e. water-permeable) seed coats. Seed mass and shape (defined by seed variance) was as measured in Chapter 2 (Table 2.3).

Species were categorised into four functional groups reflecting their dependence on post-fire seedling recruitment, as described in Chapter 2 (Table 4.1). Group 1 (post-fire recruitment essential) contains obligate seeders with persistent seedbank, local dispersal and intolerant establishment (equivalent to GI and SI species types of Noble & Slatyer 1980). Group 2 (seedling recruitment important but not critical after every fire) contains obligate seeders with wide dispersal (DI) and species with a variable fire response, persistent seedbank, local dispersal and intolerant establishment. Group 3 (seedling recruitment not essential) contains resprouters (persistent seedbank, local dispersal, intolerant; VI), as well as resprouter or variable fire response species with persistent seedbank and any of the traits of post-fire flowering (Σ), wide seed dispersal (Δ), or tolerant establishment (VT). Group 4 is the species that would not normally encounter a fire-related germination cue, resprouters with post-fire flowering and transient seedbanks (U). For the purpose of analysis groups 3 and 4 were combined here.

The relationships between the effect of heat (germination response and seed mortality) and categorical variables (functional type and seed coat) were tested with contingency tables (chi-square test of independence; Sokal & Rohlf 1987). Note that due to the small sample size, the low expected frequencies may result in a conservative test. Thus a Monte Carlo permutation method (using 1000 runs) was also used to calculate the expected distribution of χ^2 (X^2) and the associated probability by the methods of Roff & Bentzen (1980) and Zaykin & Pudovkin (1993). Species with no germination recorded in any treatment were excluded from this analysis.

The relationships between the effect of heat (germination response and seed mortality) and continuous variables (seed mass and seed variance) were analysed with one-factor ANOVAs. Species with no germination recorded in any treatment were excluded from this analysis.

Table 4.1 Details of species examined. Fire response (FR): S = obligate seeder, S r = variable fire response (facultative seeder), R = resprouter, R s = variable fire response. Functional group (FG): 1 = post-fire seedling recruitment essential, 2 = recruitment important but not critical, 3 = recruitment not essential, 4 = species without persistent seedbanks (see text for full details). Seed coat (SC) type: H = hard; S = soft. Seeds per replicate: either a known or approximate (c.) number of seeds were counted, or a chosen weight (g) measured per replicate, and average (av.) number of seeds calculated. Temperature range used: 1 = control, 60, 70, 80, 90, 100 °C; 2 = control, 60, 80, 100, 120 °C. Seed lot is defined in Chapter 2. Seed age at testing is given in months. Viability was measured via cut test, given as mean ± standard error; * where average viability from a separate test was used, only the mean is given; nm = not measured (see text for details).

Species	FR	FG	SC	Seeds per replicate	Range used	Seed lot	Seed age	Viability
Acacia suaveolens	S	1	H	15	2	a	28	96.7 ± 1.54
Actinotus helianthi	S	1	S	20	1		10	nm
Astrotricha floccosa	S?	1	H	50	2		23	nm
Bauera rubioides	R s	2	S	25	2		2	93.1 ± 1.25
Boronia ledifolia	S	1	H	25	1		8	91.7 ± 1.75
Calytrix tetragona	R s	2	S	25	2		≥9	81.8 ± 0.81
Conospermum taxifolium	R s	2	S	25	1	a	23	85.9 ± 1.30
Dillwynia retorta	S	1	H	25	2	b	17	80.4 ± 2.95
Dodonaea triquetra	S	1	H	50	1		3	97.9 ± 0.40
Doryanthes excelsa	R	4	S	25	2		≥9	nm
Epacris microphylla	S r	2	S	0.005g, av. 183	1	a	3	nm
Haemodorum planifolium	R	4	S	25	2		5	84.6 *
Hibbertia scandens	R s	3	S	25	1		≥3	65.6 ± 2.7
Kunzea ambigua	S	1	S	c. 50, av. 61	1		≥3	94.7 ± 0.61
Kunzea capitata	S r	2	S	0.01g, av. 211	1		2	nm
Lomandra longifolia	R	3	S	25	2		≥9	49.0 ± 3.59
Mitrasacme polymorpha	R s	3	S	0.005g, av. 148	1		2	nm
Ozothamnus diosmifolius	S r	2	S	c. 25, av. 26	1		≥3	84.6 ± 2.62
Patersonia glabrata	R	3	S	25	2		≥9	90.0 ± 3.46
Phebalium squamulosum	R s	2	S	25	1		3	93.1 ± 1.85
Pimelea linifolia	S r	2	S	25	1	b	12	81.8 ± 2.13
Sprengelia incarnata	S	1	S	0.01g, av. 279	1		3	nm
Telopea speciosissima	R	4	S	25	2		≥9	mm
Thelionema caespitosum	R	3	H	25	2		≥3	96.8 ± 1.16
Woollsia pungens	S r	2	S	0.02g, av. 96	1	a	11	97.9 ± 0.34
Xanthorrhoea resinifera	R	4	S	25	2		≥9	86.7 *
Zieria laevigata	S	1	H	20	1		10	78.6 ± 1.59

Results

Over the range of temperatures tested, the effect of heat-shock treatment was divided into three main categories: heat-shock had no effect on germination, heat-shock had a negative influence, and heat-shock had a positive influence (Table 4.2).

In species showing no effect of heat treatment, no germination in any treatment was recorded for five species, and low levels of germination (<10%) with no significant treatment effects on germination occurred in six species. High temperature was considered to be lethal to two of these (*Bauera rubioides* and *Pimelea linifolia*).

Where germination was reduced by heat, germination was equivalent to control levels at lower temperatures, with higher temperatures being either inhibitory (i.e. germination was significantly reduced, but mortality was not significantly increased; Fig. 4.1) or lethal (i.e. germination was significantly reduced because of heat-induced mortality; Fig. 4.2).

Where germination was increased by heat treatment, two patterns were seen. In some species germination was significantly increased above control levels at some temperatures, with a peak in germination level at an optimum temperature, followed by significantly reduced germination at higher temperatures due to heat-induced mortality (Fig. 4.3). In other species, germination was significantly increased by heat treatment with no inhibitory or lethal effects seen within the range of temperatures tested. These species either had a peak of germination at a specific optimum temperature, or a wide range of temperatures with the same influence on germination (Fig. 4.4).

A dependent relationship was found between the influence of heat on germination and functional group (Table 4.3a; $P < 0.001$). All species in functional group 1 (post-fire seedling recruitment essential) had a positive germination response, while no species from functional groups 3 and 4 (post-fire seedling recruitment not essential) had a positive response. Within this group, all three post-fire flowering species had a negative response to heat treatment.

A dependent relationship was also found between the influence of heat on germination and seed coat type (Table 4.4a; $P = 0.005$). All species with a hard seed coat had a positive response to heat treatment, while only three of the 16 species with a soft seedcoat had a positive germination response.

There was no significant difference found between the three germination response groups (no effect, negative, positive) in either seed mass (ANOVA: $n = 20$, $F = 1.081$, $P = 0.361$) or seed shape (ANOVA: $n = 21$, $F = 1.668$, $P = 0.216$).

No relationship was found between heat-induced seed mortality and either functional group (Table 4.3b; $P = 0.345$) or seed coat type (Table 4.4b; $P = 0.586$). Species with heat-induced seed mortality had significantly heavier seeds (ANOVA: $n = 20$, $F = 4.826$, $P = 0.041$), but no significant difference was found in seed shape (ANOVA: $n = 21$, $F = 1.602$, $P = 0.221$).

Table 4.2 Summary of treatment effects on germination and seed mortality. ANOVA results for treatment effects: P value given, significant ($P < 0.050$) values have been highlighted; ~ = not tested statistically (see text). Significant temperatures given: optimal temperature = temperature/s resulting in significantly higher germination than control and/or other temperatures; inhibitory temperature = temperature/s resulting in significantly lower germination than control; lethal temperature = temperature resulting in significant seed mortality, or temperature resulting in zero germination (significantly lower germination than control and/or other temperatures).

Treatment effects	Species	ANOVA (P value)		Temperature (°C)		
		Germination	Mortality	Optimal	Inhibitory	Lethal
No treatment effect						
• no germination	*Actinotus helianthi*					
	Astrotricha floccosa					
	Hibbertia scandens					
	Ozothamnus diosmifolius					
	Telopea speciosissima					
	Thelionema caespitosum					
• very low	*Bauera rubioides*	0.311	~	n/a	n/a	100
germination	*Calytrix tetragona*	0.222	0.290	n/a	n/a	n/a
	Mitrasacme polymorpha	0.060	~	n/a	n/a	~
	Patersonia glabrata	0.078	~	n/a	n/a	~
	Phebalium squamulosum	0.321	0.605	n/a	n/a	n/a
	Pimelea linifolia	0.381	~	n/a	n/a	90
Negative heat effect						
• high temperature inhibitory (Fig. 4.1)	*Conospermum taxifolium*	**0.026**	0.790	n/a	100	n/a
	Epacris microphylla	**0.007**	~	n/a	100	n/a
	Woollsia pungens	**0.001**	0.973	n/a	90	n/a
• high temperature lethal (Fig. 4.2)	*Doryanthes excelsa*	**0.000**	~	n/a	n/a	100
	Haemodorum planifolium	**0.000**	~	n/a	100	120
	Lomandra longifolia	**0.001**	**0.008**	n/a	60	100
	Xanthorrhoea resinifera	**0.000**	~	n/a	80	100
Positive heat effect						
• high temperature lethal (Fig. 4.3)	*Acacia suaveolens*	**0.000**	**0.000**	80	100	100
	Boronia ledifolia	**0.002**	**0.015**	80-90	n/a	100
	Dillwynia retorta	**0.000**	**0.000**	60-80	n/a	100
• no inhibitory or lethal effect with high temperature (Fig. 4.4)	*Dodonaea triquetra*	**0.000**	0.976	90-100	n/a	n/a
	Kunzea ambigua	**0.003**	0.550	60-100	n/a	n/a
	Kunzea capitata	**0.000**	~	90	n/a	n/a
	Sprengelia incarnata	**0.000**	~	100	n/a	n/a
	Zieria laevigata	**0.037**	0.052	90	n/a	n/a
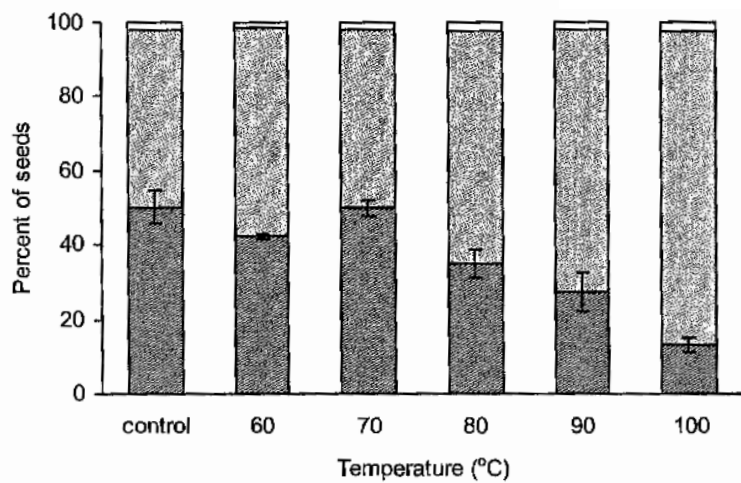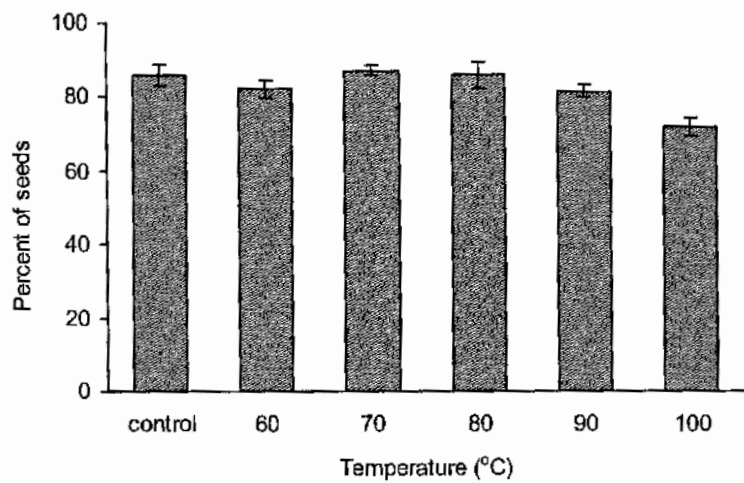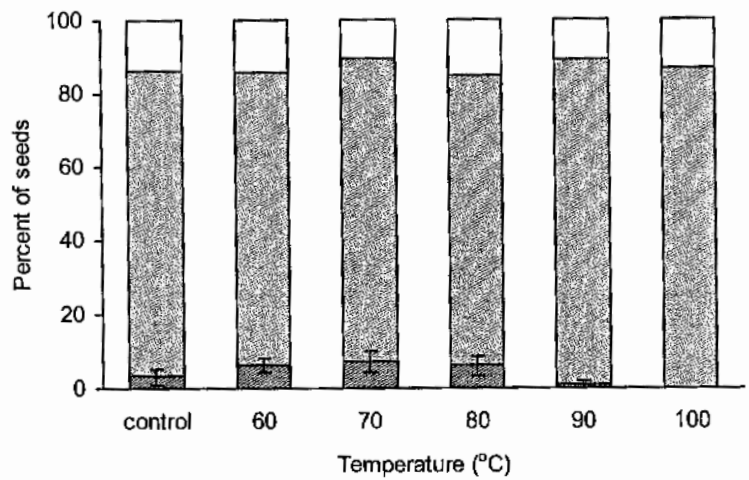
Figure 4.1 Germination responses of (a) *Conospermum taxifolium*, (b) *Epacris microphylla*, and (c) *Woollsia pungens* to heat-shock treatment. High temperature inhibitory. Dark grey bars = germinated seeds; light grey bars = ungerminated seeds; white bars = dead seeds. Error bars are standard error of percentage germination.
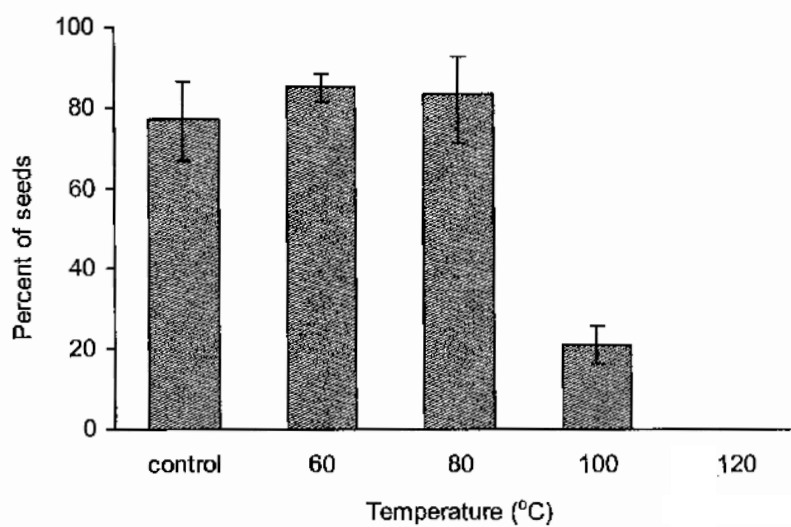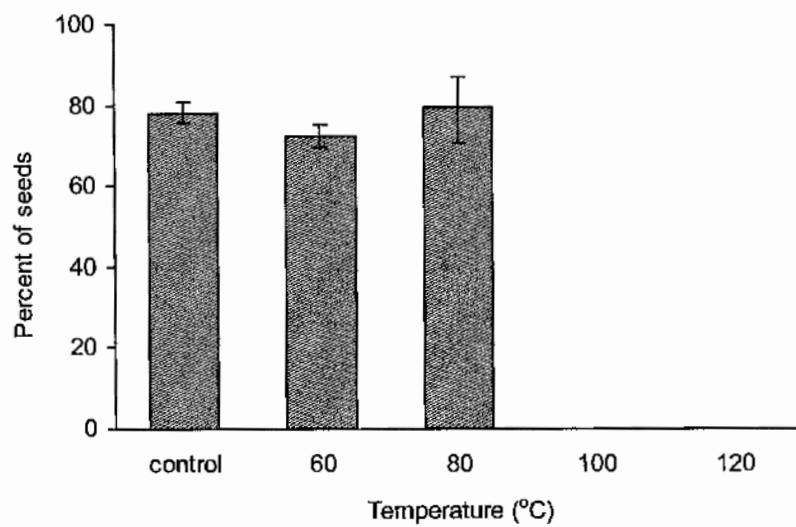
Figure 4.2 Germination responses of (a) *Doryanthes excelsa* and (b) *Haemodorum planifolium* to heat-shock treatment. High temperature lethal. Dark grey bars = germinated seed. Error bars are standard error of percentage germination.
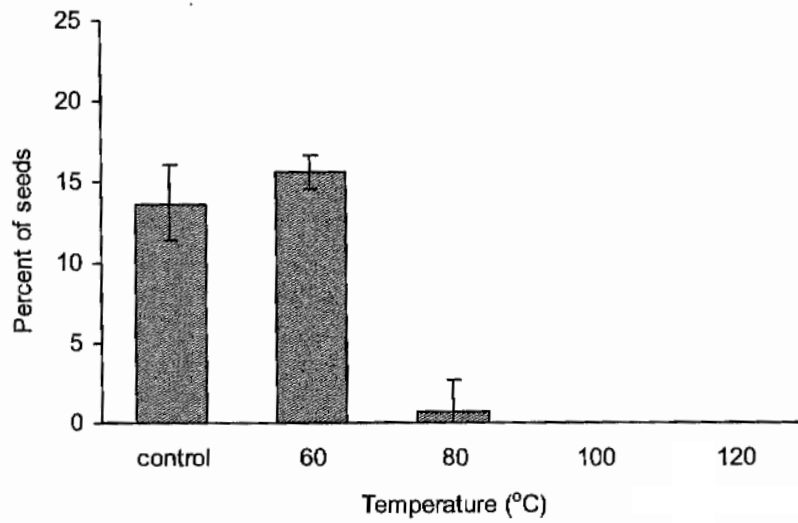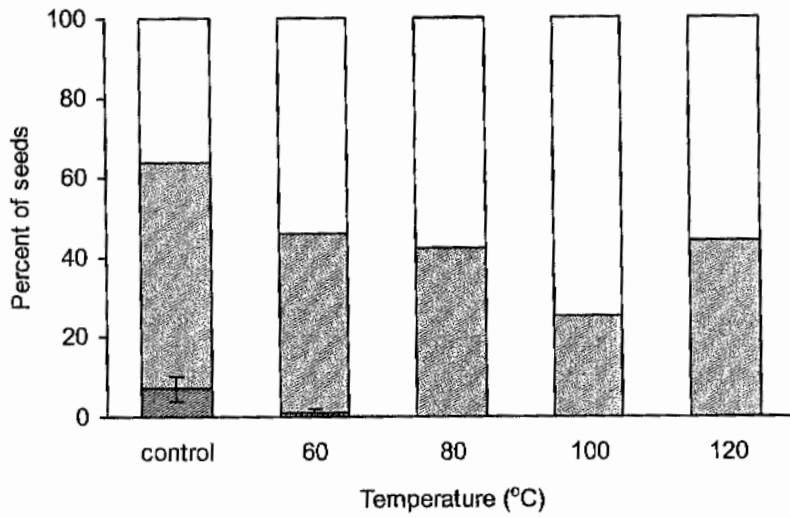
Figure 4.2 continued Germination responses of (c) *Lomandra longifolia* and (d) *Xanthorrhoea resinifera* to heat-shock treatment. High temperature lethal. Dark grey bars = germinated seeds; light grey bars = ungerminated seeds; white bars = dead seeds. Error bars are standard error of percentage germination.
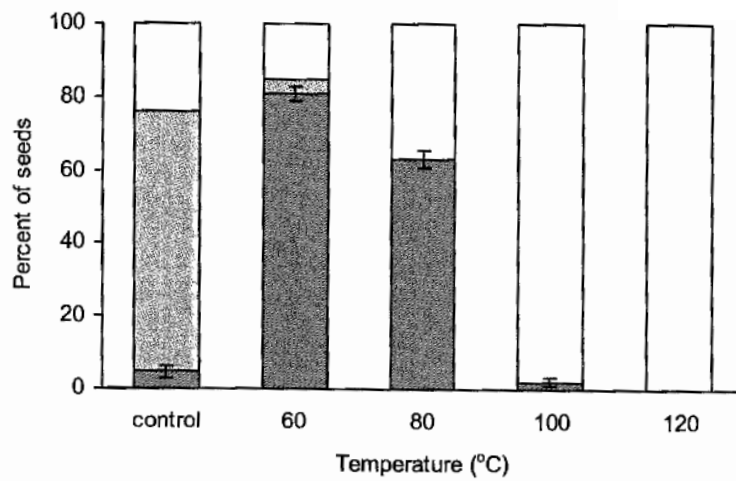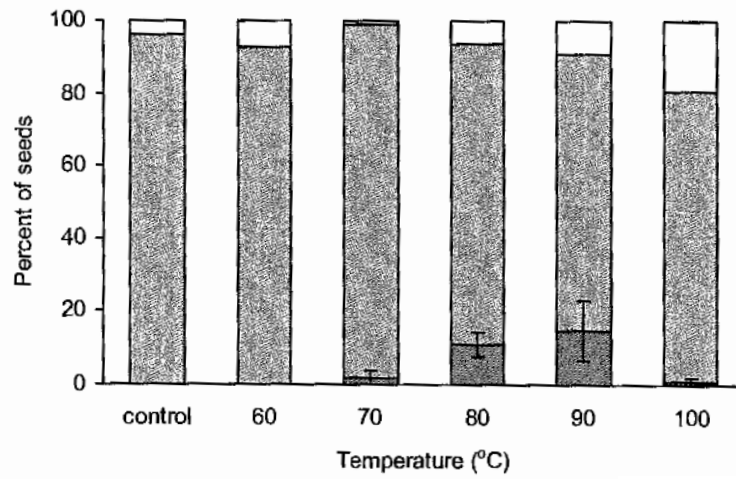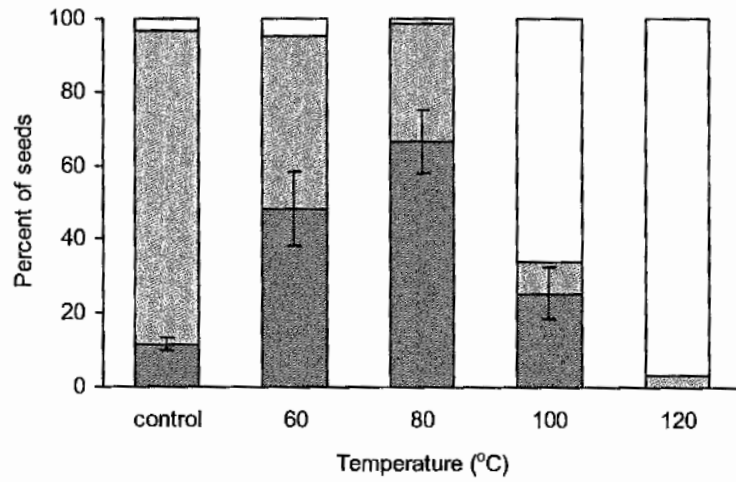
Figure 4.3 Germination responses of (a) *Acacia suaveolens,* (b) *Boronia ledifolia,* and (c) *Dillwynia retorta* to heat-shock treatment. Heat effect positive, but lethal at high temperatures. Dark grey bars = germinated seeds; light grey bars = ungerminated seeds; white bars = dead seeds. Error bars are standard error of percentage germination.
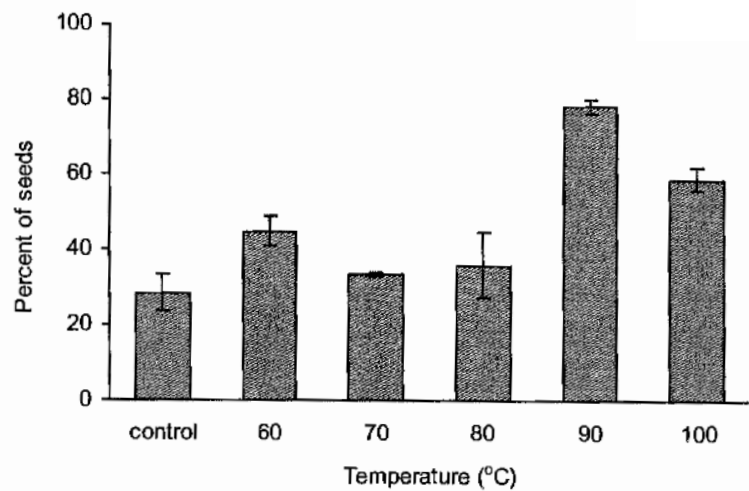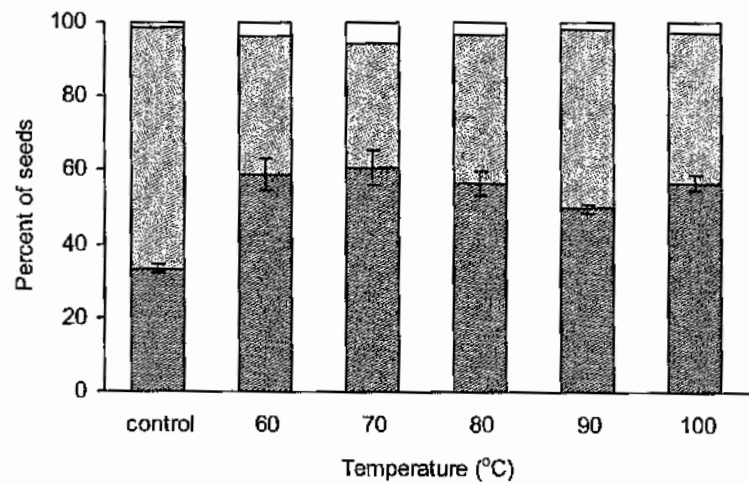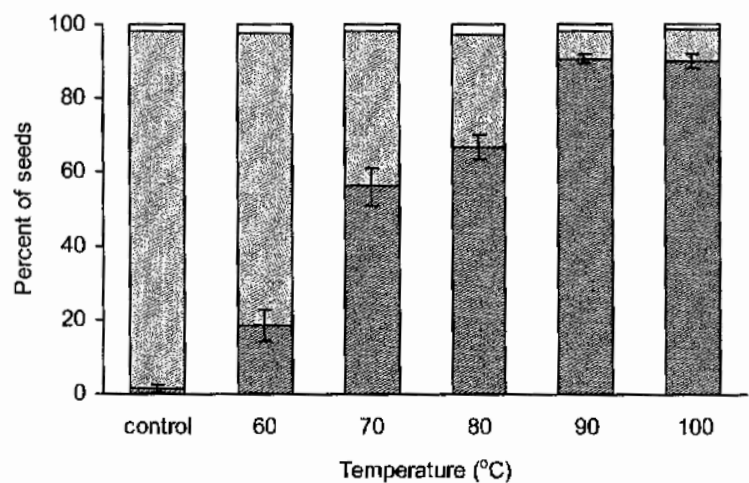
Figure 4.4 Germination responses of (a) *Dodonaea triquetra,* (b) *Kunzea ambigua,* and (c) *Kunzea capitata* to heat-shock treatment. Heat effect positive. Dark grey bars = germinated seeds; light grey bars = ungerminated seeds; white bars = dead seeds. Error bars are standard error of percentage germination.
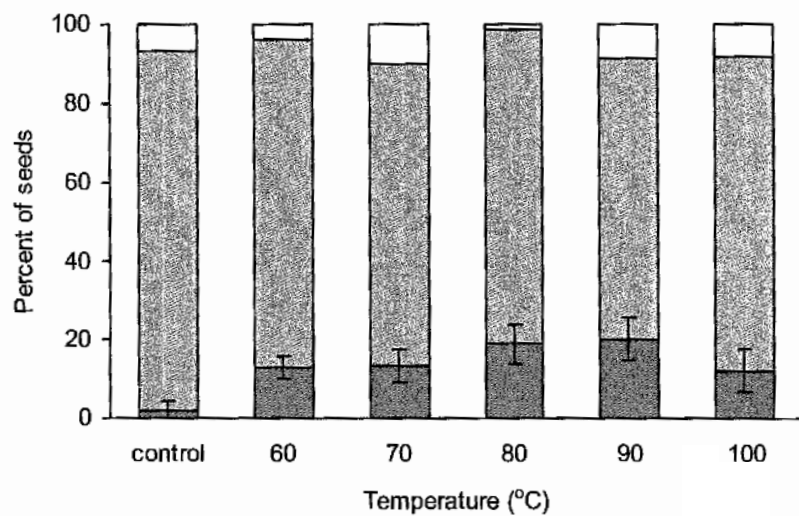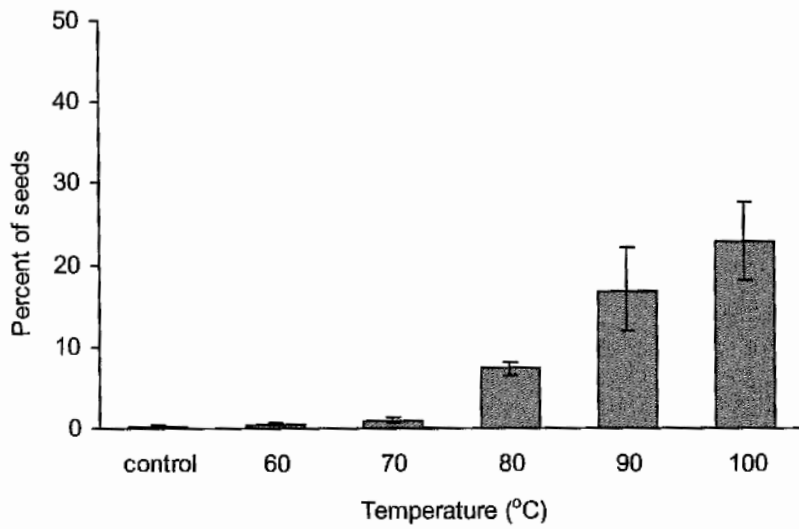
Figure 4.4 continued Germination responses of (d) *Sprengelia incarnata* and (e) *Zieria laevigata* to heat-shock treatment. Heat effect positive. Dark grey bars = germinated seeds; light grey bars = ungerminated seeds; white bars = dead seeds. Error bars are standard error of percentage germination.

Table 4.3 Relationship between functional group and (a) germination response to heat-shock treatment, and (b) heat-induced seed mortality. Contingency table: number of species in each group shown, expected values based on the null hypothesis of independence are shown in parentheses. Functional groups: 1 = post-fire seedling recruitment essential, 2 = recruitment important but not critical, 3 = recruitment not essential (including 4 = species without persistent seedbanks); see text for full details.

Heat effect	Functional group			Chi-square test:
	1	2	3 & 4	$df = 4$, .05 level, R: $\chi^2 \geq 9.488$
no effect	0 (2.0)	4 (2.3)	2 (1.7)	$\chi^2 = 18.411$, $P = 0.001$
negative	0 (2.3)	3 (2.7)	4 (2.0)	**Monte Carlo permutation:**
positive	7 (2.7)	1 (3.0)	0 (2.3)	$X^2 = 18.412$, $P < 0.001$

Lethal effect	Functional group			Chi-square test:
	1	2	3 & 4	$df = 2$, .05 level, R: $\chi^2 \geq 5.991$
yes	3 (3.0)	2 (3.4)	4 (2.6)	$\chi^2 = 2.431$, $P = 0.297$
no	4 (4.0)	6 (4.6)	2 (2.6)	**Monte Carlo permutation:**
				$X^2 = 2.431$, $P = 0.345$

Table 4.4 Relationship between seed coat type and (a) germination response to heat-shock treatment, and (b) heat-induced seed mortality. Contingency table: number of species in each group shown, expected values based on the null hypothesis of independence are shown in parentheses.

Heat effect	Seed coat		Chi-square test:
	Hard	Soft	$df = 2$, .05 level, R: $\chi^2 \geq 5.991$
no effect	0 (1.4)	6 (4.6)	$\chi^2 = 10.664$, $P = 0.005$
negative	0 (1.7)	7 (5.3)	**Monte Carlo permutation:**
positive	5 (1.9)	3 (6.1)	$X^2 = 10.664$, $P = 0.005$

Lethal effect	Seed coat		Chi-square test:
	Hard	Soft	$df = 1$, .05 level, R: $\chi^2 \geq 3.841$
yes	3 (2.1)	6 (6.9)	$\chi^2 = 0.787$, $P = 0.375$
no	2 (2.9)	10 (9.1)	**Monte Carlo permutation:**
			$X^2 = 0.788$, $P = 0.586$

Discussion

The species that were unaffected by heat treatment had very low levels of germination (<10%) in both control and treated seeds, implying that either the trial conditions were unsuitable for the species' germination requirements, or that the treatment failed to break the seed dormancy. Except for *Phebalium squamulosum*, these species have all been shown in other trials to have their dormancy broken by another fire-related cue, smoke (Chapters 3 and 5).

The species which failed to germinate at all would also have either not had their dormancy broken or been in unsuitable conditions. Four of these species (*A. floccosa, H. scandens, O. diosmifolius*, and *T. caespitosum*) have also failed to germinate in other trials (Chapters 3 and 5), and as seeds appeared to be viable they may have very strict germination requirements which were not met here.

The species whose germination was reduced by heat were generally unaffected by temperatures at the lower end of the range (60-80 °C). Higher temperatures (80-120 °C), however, reduced germination below control levels with an inhibitory or lethal response, indicating that their seed structure is sensitive to high levels of heat.

Three of these species (*Doryanthes excelsa, Haemodorum planifolium*, and *Xanthorrhoea resinifera*) are pyrogenic flowerers, which resprout and are stimulated to flower rapidly after fire, releasing non-dormant seeds to germinate immediately in the post-fire environment. They do not have a soil seedbank available prior to the fire, and hence have no reason to be adapted to heat or other fire-related germination cues. Other species without persistent soil seedbanks (transient seedbank with pyrogenic flowering, transient seedbank with wide seed dispersal, serotinous seedbank) have been shown elsewhere to be sensitive to heat treatments (Keeley 1987, Bell & Williams 1998).

The other four species with germination reduced by heat do possess persistent soil seedbanks, but do not have the hard seed coats typically associated with a heat-shock germination response. All of these species except *Lomandra longifolia* have been shown in other trials to respond to a smoke cue, and two of them (*Conospermum taxifolium, Epacris microphylla*) respond to a combined heat and smoke treatment (Chapter 5), at a temperature of 80 °C, which here had no significant effect. *Lomandra longifolia* has been described by several observers (Purdie & Slatyer 1976, Wark *et al.* 1987, Benwell 1998) as an obligate resprouter, i.e. no seedlings have been observed post-fire. The extreme heat sensitivity displayed here, with all temperatures used resulting in either inhibition of germination or seed mortality, may explain this lack of post-fire germination.

The species with heat-stimulated germination represented both hard and soft seed coat types. While the action of heat on species with water-impermeable (hard) seed coats has been thoroughly studied (e.g. Martin *et al.* 1975, Auld & O'Connell 1991, Cocks & Stock 1997, Herranz *et al.* 1998), the positive influence of heat-shock on other soil-stored seed types has rarely been examined. There is some evidence of this phenomenon from South African fynbos species. Brits *et al.* (1999) have shown how water-permeable but oxygen-impermeable seeds of *Leucospermum* species respond to desiccation-scarification effects arising from both heat-shock related to fire and repeated desiccation/hydration cycles in soils under high ambient temperatures.

Heat-stimulated germination occurred within the range 60-100 °C, with 80-90 °C being the most common peak temperature. Within the open forest vegetation of the study area, fire is required to reach this sort of temperature within the soil profile. Soil temperatures in unburnt vegetation in summer are around 20 °C, up to a maximum of 30 °C (40 °C at the soil surface) (Auld & Bradstock 1996). Under experimental fires in the area, soil temperatures of around 60 °C have been recorded (Beadle 1940, Bradstock & Auld 1995), while temperatures above 90 °C are only likely under severe fire conditions (Beadle 1940).

Two broad patterns of heat-stimulated germination were observed: step-wise germination increase with increasing temperature or peak germination at an optimal temperature followed by inhibitory or lethal effects. Even for species with heat-stimulated germination, death is expected at excessive temperatures (generally 120-150 °C; Keeley 1991) and interactions between temperature and duration of heating become important (Auld & O'Connell 1991, Cocks & Stock 1997).

These different patterns in heat response and optimal temperature mean that a wide variety of reactions to one temperature is seen between the different species. While some species have a very broad heat response others have much more specific temperature requirements. Having a wide range of stimulatory temperatures would allow a species either to have seeds germinate from a variety of depths, or always have some seeds germinate under variable heating conditions (Herranz et al. 1998). It has been suggested that for co-occurring species it is advantageous to have different optimal temperatures in order to avoid high levels of seedling competition (Trabaud & Oustric 1989). With the spatial variability that occurs in fire conditions and soil heating (Christensen & Kimber 1975, Atkins & Hobbs 1995), the various heat responses displayed here should allow some seeds of each species to find their optimal germination requirements, and thus community diversity will be maintained.

Barro & Poth (1988) and Moreno & Oechel (1991) have shown that seeds of seeder species are more tolerant of higher temperatures than those of resprouters, hypothesising that seeds of obligate seeders are thus better adapted to survive fire. However, in this study heat-induced seed mortality was not found to be related to either functional group or seed coat type. The two studies finding a significant mortality relationship with fire response were both conducted on a single congeneric pair of species. Bell & Williams (1998) found a general pattern of greater heat-induced seed mortality in resprouters, but the variation involved when examining several species together made the pattern statistically insignificant.

A pattern in seed mortality was found here in relation to seed size, with more large-seeded species having a lethal effect at high temperature. Most studies that have examined this relationship have found the opposite (Valbuena et al. 1992, Gonzalez-Rabanal & Casal 1995, Gashaw & Michelsen 2002), however Hanley et al. (2003) have recently also found smaller seeds to be more heat tolerant. This relationship is probably more complicated than simple seed size, for example the relative thickness of the seed coat to the embryo affects heat tolerance (Cocks & Stock 1997).

Unlike seed mortality, the overall germination response to heat-shock was found to be related to the defined functional groups. All species with heat-stimulated germination were obligate or facultative seeders, where the adult plants are killed by fire (Kunzea capitata has some ability to resprout dependent on the fire intensity experienced; personal observation). They are thus dependent on post-fire seedling recruitment for maintenance of the population and would be expected to be well adapted to a fire-related

germination cue. For the species with a hard seed coat (*A. suaveolens, D. retorta, D. triquetra*, and to a lesser extent *B. ledifolia* and *Zieria laevigata*) it is expected that the key germination cue would be heat, as the effect of heat on water-impermeable seed coats has been well demonstrated (Keeley 1991).

It should be noted that the only hard-seeded resprouter species tested (*T. caespitosum*) failed to germinate, so the lack of hard-seeded vegetative regenerators may have skewed the analysis, especially considering that there was also a relationship found between heat response and seed coat type. An examination of the fire response of hard-seeded families within NSW (Table 4.5) shows that a much greater proportion of species with hard seed coats are seeders than resprouters. Given that a hard seed coat is a reliable indicator of responding to a heat cue (Table 4.4a; Auld and O'Connell 1991, Bell *et al.* 1993), the hypothesis that seeders have a stronger response to heat-shock as a germination cue seems reasonable.

Those species with germination either unaffected or decreased by heat treatment were all species considered to be less reliant on post-fire seedling recruitment (resprouters and facultative seeders). Although these species still need to recruit seedlings into their populations (i.e. to replace individuals lost to senescence as well as mortality related to fire and other damage), it is not as imperative as it is for obligate seeder species. While the advantages of establishment in the post-fire environment still hold for resprouters, they tend to have lower levels of seedling establishment than seeders irrespective of comparative seed production (Keeley 1977). This may be because their seeds are not as strongly adapted to a fire-related stimulus as are those of seeder species. This is also displayed by the negative heat response shown by all the species that would not normally encounter a heat-shock cue.

Table 4.5 Proportion of obligate seeder to vegetative regenerator species within families containing hard seed coats in NSW, Australia. All species occurring within NSW for which fire response information was available (NSW Flora Fire Response Database; Kenny & Bradstock 2001) were tallied. References to hard-seeded quality of family: 1= Auld & O'Connell (1991), 2 = Keeley (1991), 3 = Baskin & Baskin (1989). Families represented in this study: Fabaceae, Rutaceae, Sapindaceae.

Family	Total number of species	Ratio OS:VR
Convolvulaceae [2,3]	6	2:1
Fabaceae [1]	291	2:1
Lamiaceae [1]	28	4.6:1
Malvaceae [3]	14	2.5:1
Rhamnaceae [1,2,3]	28	3:1
Rutaceae	69	1.9:1
Sapindaceae [2]	17	2.4:1

Outcome

From this chapter, heat application for further experiments (Chapter 5) was set at 80 °C for 10 minutes. This was shown here and elsewhere (Auld & O'Connell 1991) to be within the optimum temperature range for heat-shock effects on many species. Higher temperatures were shown to be inhibitory or lethal for many of the species tested.

CHAPTER 5: MULTIPLE GERMINATION CUES

Aim

The effects of the fire-related germination cues of heat and combustion products (smoke and charred wood) have been studied on numerous plant taxa in several regions of the world. However, in NSW heat has only been studied in legume species, and few species have been shown to have a smoke cue. These different cues have rarely been studied in combination, and it has been suggested that heat and smoke are complementary germination triggers, acting on different species within the soil seedbank (Read *et al.* 2000, Enright & Kintrup 2001). However, it has recently been shown that some species respond to both heat and smoke cues (Keith 1997, Gilmour *et al.* 2000, Morris 2000).

This chapter investigates the individual and combined influence of the fire-related germination cues heat, smoke and charred wood on 35 species representing a range of different seed morphologies and species life histories. The effects of these cues on germination are then related to the species' fire response and discussed in terms of the role of fire in population persistence.

As seeder species are reliant on seedling recruitment alone for population persistence after a fire, it is predicted that seeder species should exhibit greater post-fire seedling establishment than resprouter species (Keeley 1977). This could be achieved by higher levels of seed production, germination or seedling survival (Moreno & Oechel 1992). For higher germination levels to occur it might be expected that seeders should have a stronger response to fire-related germination cues.

Methods

Treatment Methods

Three fire-related germination cues (heat, smoke and charred wood) were tested for their individual and combined influence on the germination of 35 species (39 seed lots). See Table 5.1 for study species.

Heat treatment was applied to loose seeds in an oven pre-heated to 80 °C for 10 minutes. This temperature was chosen as both simulating conditions in the seedbank (upper 5 cm of the soil profile) under the passage of fire (Bradstock & Auld 1995), and being within the optimum temperature range for heat-shock effects on many species (Auld & O'Connell 1991, Chapter 4). Higher temperatures than this have been shown to be lethal for many of the study species (Chapter 4).

Smoke was produced by burning dried litter material from the vegetation community of the test species (open woodland with *Eucalyptus* and *Banksia* species as canopy dominants, Ku-ring-gai Chase National Park) in a beekeeper's burner. The smoke was channelled into a chamber containing the seeds through a length of hose sufficient to cool the smoke (Brown 1993b). Seeds were fumigated by smoke for 15 minutes (Chapter 3).

Two methods of charred wood application were utilised throughout these trials; which method was applied to each species is indicated in Table 5.1. Originally charred wood was produced by heating wood from *Eucalyptus haemastoma* in a muffle furnace until fully charred but not ashed. The charred wood was ground, then 0.05 g of this material was scattered over the seeds in the Petri dish. While most published experiments with charred wood have used more charate than this, lower concentrations, equivalent to

those used here, can be as effective (Keeley & Pizzorno 1986, Brown 1993a). In later trials, charred wood was produced using the methods of Wicklow (1977). Stem segments (<1 cm diameter) of *Eucalyptus haemastoma* were placed in a 30 cc crucible and heated over a bunsen burner flame until charred (approximately 9 minutes) then the lid placed over the crucible to stop combustion. The charred stems were then finely ground in a mortar and pestle, and this material used to produce a 10 g/l solution by placing 1 g of charate in 100 ml distilled water. This was agitated thoroughly and left to stand for 60 hours before filtering through a 0.5 mm mesh.

The three treatments (heat, smoke and charred wood) were applied to all species in orthogonal combinations. For seed batches receiving combined treatments, these were applied in the order heat, smoke, then charred wood. For some species only heat and smoke were tested as there were fewer available seeds. For each treatment four replicates were treated independently (Morrison & Morris 2000), i.e. replicate seed batches heated and smoked at separate times; four batches of charate produced separately.

Germination

Each treatment was performed on four replicate seed batches. Each replicate seed batch consisted of either a known (usually 25) or approximate number of seeds (Table 5.1). For those where approximate numbers were used (very small seeded species) the total number of viable seeds per batch was calculated at the end of the trial.

Following treatment, seeds were placed in Petri dishes lined with Whatman No. 1 filter paper and watered with distilled water. Dishes were re-watered as required, and periodically checked for germination. Germination was determined as being when the radicle emerged, and germinated seeds were removed from the Petri dish. Dishes were kept under ambient laboratory conditions in a dark cabinet (but checked in light). Trials ran until no further germination was recorded for at least 1 week (trial period of 8-10 weeks, unless a longer time was required for commencement of germination).

At the end of each trial seed viability was assessed via a cut test. Each seed was cut open to expose the embryo, and graded as viable (healthy, plump, white embryo), dead or empty. Germination was expressed either as a percent of the number of viable seeds available per replicate, or (where treatment caused seed mortality) as a percent of the mean number of viable seeds calculated on a sub-set of the total replicates. In species for which the cut test was difficult to perform and/or inconclusive, germination is expressed as percentage of total seeds available. Viability is given in Table 5.1.

Percentage germination data were arcsine transformed to improve normality and homogeneity (Sokal & Rohlf 1987). Back-transformed data are presented in figures. Homogeneity of variance was then checked by Cochran's test. For each species, germination level data were analysed using a three-factor orthogonal analysis of variance (ANOVA) to assess the treatment effects (heat, smoke and charred wood) and their interactions. For species where charred wood was not utilised, data were analysed with a two-factor ANOVA.

Table 5.1 Details of species examined. Fire response (FR): S = obligate seeder, S r = variable fire response (facultative seeder), R = resprouter, R s = variable fire response. Functional group (FG): 1 = post-fire seedling recruitment essential, 2 = recruitment important but not critical, 3 = recruitment not essential, 4 = species without persistent seedbanks (see text for full details). Number of seeds per replicate is given, for smaller seeds an approximate number of seeds were used and then the average (av.) number calculated. Method of charred wood application indicated; charate (0.05 g), solution (10 g/l) or no charred wood used. Seed lot: see Chapter 2 for details. Seed age (from collection or purchase) when tested is given in months. Viability was measured via cut tests, given as mean ± standard error; nm = not measured (see text for details).

Species	FR	FG	Seeds / replicate	Charred wood applied	Seed lot	Seed age	Seed viability
Acacia suaveolens	S	1	25	charate	a	2	96.3 ± 0.76
Acacia terminalis	S r	2	20	charate		10	99.4 ± 0.30
Actinotus minor	S r	2	25	charate		≥8	nm
Astrotricha floccosa	S?	1	50	charate		28	nm
Baeckea imbricata	R	3	25	solution		15	nm
Bauera rubioides	R s	2	25	solution		30	92.9 ± 1.73
Boronia ledifolia	S	1	25	charate		9	96.6 ± 0.68
Calytrix tetragona	R s	2	25	solution		≥11	96.8 ± 0.71
Cassytha pubescens	S	2	20	none		28	nm
Conospermum taxifolium	R s	2	25	solution	a	28	88.8 ± 1.90
Conospermum taxifolium	R s	2	30	solution	b	4	85.0 ± 0.96
Dianella caerulea	R	4	25	solution		≥8	95.3 ± 1.23
Dianella revoluta	R	4	25	solution		≥8	98.6 ± 0.61
Dillwynia retorta	S	1	15	charate	a	9	79.2 ± 1.91
Dodonaea triquetra	S	1	50	charate		2	97.1 ± 0.39
Epacris microphylla	S r	2	av. 120	solution	a	16	97.0 ± 0.40
Epacris microphylla	S r	2	av. 69	solution	b	4	96.6 ± 0.45
Eriostemon australasius	S r	2	15	charate	a	9	90.0 ± 1.23
Gahnia sieberiana	R	3	25	solution		17	nm
Grevillea buxifolia	S r	2	14	none	a+b	5-17	90.4 ± 1.36
Grevillea sericea	S r	2	25	charate	a	11	71.9 ± 2.42
Grevillea sericea	S r	2	25	charate	b	8	99.4 ± 1.34
Grevillea speciosa	S r	2	20	charate	a	8	98.5 ± 0.46
Haemodorum planifolium	R	4	25	charate		9	nm
Hibbertia scandens	R s	3	25	solution		≥8	76.0 ± 2.83
Kunzea ambigua	S	1	av. 125	solution		≥11	99.1 ± 0.21
Kunzea capitata	S r	2	av. 56	solution		15	95.4 ± 0.54
Lasiopetalum ferrugineum	R s	2	25	charate		3	91.0 ± 1.45
Lomandra longifolia	R	3	25	solution		≥11	81.5 ± 1.67
Mitrasacme polymorpha	R s	3	av. 70	solution		18	91.6 ± 0.94
Patersonia glabrata	R	3	25	solution		≥8	60.7 ± 3.53
Persoonia pinifolia	S	2	25	solution		21	nm
Phebalium squamulosum	R s	2	25	solution		19	82.3 ± 1.51
Pimelea linifolia	S r	2	15	none	a	31	88.1 ± 2.21
Pimelea linifolia	S r	2	25	solution	b	20	81.4 ± 1.61
Sprengelia incarnata	S	1	av. 102	solution		17	c. 100
Thelionema caespitosum	R	3	25	solution		≥11	94.0 ± 1.16
Woollsia pungens	S r	2	av. 68	solution	a+b	20-32	74.3 ± 1.70
Zieria laevigata	S	1	25	charate		2	nm

Germination response was categorised based on the main treatment effects and interactions. The potential combinations of treatment effects are listed in Table 5.2. Species were placed in these categories based on the outcome of the ANOVA and the germination level per treatment.

Seed Lot Comparisons

For a few species two seed lots were collected from the same location in different years (*Conospermum taxifolium, Epacris microphylla, Pimelea linifolia*) or different seasons (*Grevillea sericea*). For these species, germination trials were performed on the two seed lots at the same time, such that the older seed lot was stored for a longer period of time, but the conditions during the trial were identical.

Onset of Germination

For the *Grevillea* species, germination was scored three times per week in order to asses the time taken to begin germination. Onset of germination was measured as the number of days taken for the first seed within a replicate batch to germinate. When no germination occurred within a replicate this value was treated as 56 days (the period of the trial). Data were analysed using a two (*Grevillea buxifolia*, no charred wood treatments) or three (*Grevillea sericea* and *Grevillea speciosa*) factor orthogonal ANOVA. Prior to ANOVA, data were assessed for homogeneity of variance using Cochran's test, and a log transformation was applied to data for *G. sericea* and *G. speciosa*.

Functional Groups

Species were categorised into four functional groups reflecting their dependence on post-fire seedling recruitment, as described in Chapter 2. Group 1 (post-fire recruitment essential) contains obligate seeders with persistent seedbank, local dispersal and intolerant establishment (equivalent to GI and SI species types of Noble & Slatyer 1980). Group 2 (seedling recruitment important but not critical after every fire) contains obligate seeders with wide dispersal (DI) and species with a variable fire response, persistent seedbank, local dispersal and intolerant establishment. Group 3 (seedling recruitment not essential) contains resprouters (persistent seedbank, local dispersal, intolerant; VI), as well as resprouter or variable fire response species with persistent seedbank and any of the traits of post-fire flowering (Σ), wide seed dispersal (Δ), or tolerant establishment (VT). Group 4 is the species that would not normally encounter a fire-related germination cue, resprouters with transient seedbanks and rapid post-fire flowering (U). For the purpose of analysis groups 3 and 4 were combined here.

The relationship between germination response and the functional groups was tested with a contingency table (chi-square test of independence; Sokal & Rohlf 1987). The germination response categories of Table 5.3 and the functional groups described above were used (listed in Table 5.1). Note that due to the small sample size, the low expected frequencies may result in a conservative test. Thus a Monte Carlo permutation method (using 1000 runs) was also used to calculate the expected distribution of χ^2 (X^2) and the associated probability by the methods of Roff & Bentzen (1980) and Zaykin & Pudovkin (1993). Species that failed to germinate at all were not included in this analysis.

Table 5.2 Potential response to multiple germination cues. In this scenario the two combustion products, smoke and charred wood, are treated as equivalent. The individual effects of heat and combustion product are potentially: not significant (ns), negative (-), or positive (+). This will be reflected in the ANOVA by which terms are significant. The potential combinations of treatment effects are: none significant (ns), germination less than individual effects, equal to individual effects, or greater than individual effects. This will be reflected by the germination levels and in the ANOVA by the interaction term.

Germination response	Heat effect	Combustion effect	Main effect term significant	Combination of heat and combustion	heat × combustion interaction
No treatment effects	not significant	not significant	none	not significant	not significant
Heat inhibitory or lethal	negative	not significant	heat	not significant	not significant
Heat effect only	positive	not significant	heat	not significant	not significant
Heat effect, combustion inhibitory	positive	negative	heat, combustion	less than individual effect	not significant
Heat effect, combustion lethal	positive	negative	heat, combustion	less than individual effect	significant
Combustion inhibitory or lethal	not significant	negative	combustion	not significant	not significant
Combustion effect only	not significant	positive	combustion	not significant	not significant
Combustion effect, heat inhibitory	negative	positive	heat, combustion	less than individual effect	not significant
Combustion effect, heat lethal	negative	positive	heat, combustion	less than individual effect	significant
Heat and combustion equal	positive	positive	heat, combustion	same as individual effects	significant
Heat and combustion additive	positive	positive	heat, combustion	sum of two effects	not significant
Heat and combustion synergistic	positive	positive	heat, combustion	greater than sum of two effects	significant
Heat and combustion unitive	positive	positive	heat, combustion	effect only with combination	significant

Results

Germination Response

Viability was high for most species tested. Control germination level ranged from 0% (*Boronia ledifolia, Dianella caerulea, Grevillea buxifolia, Gahnia sieberiana, Pimelea linifolia* (seed lot a)) to 98% (*Haemodorum planifolium*). Germination level after the optimum treatment ranged from 4% (*Pimelea linifolia* (seed lot b), *Zieria laevigata*) to 100% (*Dillwynia retorta*).

A wide variety of treatment effects was observed (Table 5.3). Of the 35 species (39 seed lots) tested, five species failed to germinate under any treatment conditions (*Actinotus minor, Astrotricha floccosa, Baeckea imbricata, Hibbertia scandens* and *Thelionema caespitosum*), another ten showed no treatment effects, seven responded only to heat, four only to smoke, and thirteen to both heat and smoke. Of the species showing no treatment effects, eight had very low germination (<5%) across all treatments, *Haemodorum planifolium* (Fig. 5.1a) showed no dormancy, and *Lomandra longifolia* (Fig. 5.1b) had a lethal response to heat.

Charred wood alone had no significant effect on any species, but did interact with the other cues in a few cases. For *Boronia ledifolia* these interactions were inconsistent (germination was higher with heat alone compared to heat and charred wood, while germination was higher with heat, smoke and charred wood all combined in comparison to heat and smoke without charred wood). Charred wood slightly increased the germination of *Calytrix tetragona* only in the absence of smoke, while germination of *Epacris microphylla* (seed lot a), *Eriostemon australasius* and *Lasiopetalum ferrugineum* was slightly increased by charred wood only in the presence of heat. In *Persoonia pinifolia* smoke only increased germination in the absence of charred wood.

Both smoke and heat cues had a strong influence on many species, with some species having an additive or interactive relationship between the two cues. The influence of smoke was only ever positive, while heat had a negative effect on a few species. Heat was lethal to *Lomandra longifolia*, where no seeds germinated in heated treatments and seed viability was significantly ($P < 0.001$) reduced from 82% in unheated treatments to 23% when heated. Heat was also lethal or inhibitory to three species with a smoke response (*Bauera rubioides, Pimelea linifolia, Woollsia pungens*), counteracting the positive smoke influence when the two cues were combined.

The various observed responses were categorised (as per Table 5.2) into four main treatment effects (Table 5.3): no treatment effect, heat response only (Fig. 5.2), smoke response only (Fig. 5.3), and combined heat and smoke response. A variety of combined heat and smoke responses was seen (Table 5.3). Heat and smoke and the two combined can produce equal levels of germination (Fig. 5.4). The heat and smoke effects can be equal and additive (Figs 5.5a-c) or unequal and additive (Figs 5.5d-f); for these species, the greatest germination is achieved when the two cues are combined. Heat and smoke may have no individual effects but act in unison to increase germination (Fig. 5.6). For *Eriostemon australasius* (Fig. 5.6b), germination was further restricted to the occurrence of all three cues (heat, smoke and charred wood).

Table 5.3 Summary of main treatment effects on germination level. Seed lot is differentiated (a, b; see Chapter 2 for details) for those species where 2 seed lots were tested. Results of 3-factor ANOVA: P value given for each factor (charred wood, smoke, heat), significant ($P < 0.050$) values have been highlighted; significant ($P < 0.050$) interactions are listed (s = smoke, c = charred wood, h = heat). n/a = charred wood not tested; ns = no significant interactions.

Treatment effects	Species with this response	Charate	Smoke	Heat	Interactions
No treatment effects					
• no germination	*Actinotus minor*				
	Astrotricha floccosa				
	Baeckea imbricata				
	Hibbertia scandens				
	Thelionema caespitosum				
• very low germination (<5%)	*Cassytha pubescens*	n/a	0.382	0.753	ns
	Conospermum taxifolium a	0.117	0.669	0.669	ns
	Dianella caerulea	0.864	0.072	0.069	ns
	Dianella revoluta	0.609	0.230	0.847	ns
	Patersonia glabrata	0.598	0.271	0.335	ns
	Phebalium squamulosum	0.596	0.269	0.860	ns
	Persoonia pinifolia	0.053	0.142	0.990	s×c
	Pimelea linifolia a	n/a	0.529	0.856	ns
• no dormancy (Fig. 5.1a)	*Haemodorum planifolium*	0.231	0.171	0.900	ns
• heat lethal (Fig. 5.1b)	*Lomandra longifolia*	0.259	0.308	**0.001**	ns
Heat effect only					
• increased germination with heat; smoke no significant effect (Fig. 5.2a-g)	*Acacia suaveolens*	0.264	0.702	**0.000**	ns
	Acacia terminalis	0.826	0.556	**0.000**	ns
	Boronia ledifolia	0.583	0.601	**0.000**	s×c, s×c×h
	Dillwynia retorta	0.441	0.704	**0.000**	ns
	Dodonaea triquetra	0.527	0.400	**0.000**	ns
	Lasiopetalum ferrugineum	0.588	0.080	**0.000**	c×h
	Zieria laevigata	0.563	0.994	**0.001**	ns
Smoke effect only					
• germination increased with smoke; heat no significant effect (Fig. 5.3a)	*Calytrix tetragona*	0.196	**0.000**	0.528	s×c
• germination increased with smoke; heat lethal or inhibitory (Fig. 5.3b-d)	*Bauera rubioides*	0.508	**0.008**	**0.000**	ns
	Pimelea linifolia b	0.345	**0.015**	**0.000**	s×h
	Woollsia pungens	0.563	**0.001**	**0.001**	ns
Combined heat and smoke effect					
• heat and smoke equal: germination equally increased with heat, smoke, heat + smoke (Fig. 5.4a-d)	*Epacris microphylla* a	0.610	**0.000**	**0.000**	s×h
	Epacris microphylla b	0.791	**0.000**	**0.000**	s×h, c×h
	Kunzea ambigua	0.526	**0.000**	**0.000**	s×h
	Sprengelia incarnata	0.133	**0.000**	**0.000**	s×h
• heat and smoke additive: germination increase greatest when heat and smoke combined (Fig. 5.5a-f)	*Gahnia sieberiana*	0.864	**0.006**	**0.004**	ns
	Grevillea buxifolia	n/a	**0.000**	**0.003**	ns
	Grevillea sericea a	0.834	**0.000**	**0.001**	ns
	Grevillea sericea b	0.215	**0.000**	**0.000**	ns
	Grevillea speciosa	0.385	**0.000**	**0.003**	ns
	Kunzea capitata	0.788	**0.000**	**0.000**	ns
• heat and smoke unitive: germination increased only when heat and smoke combined (Fig. 5.6a-d)	*Conospermum taxifolium* b	0.890	**0.037**	**0.000**	s×h
	Eriostemon australasius	0.774	**0.013**	0.078	c×h
	Mitrasacme polymorpha	0.346	**0.001**	0.070	s×h
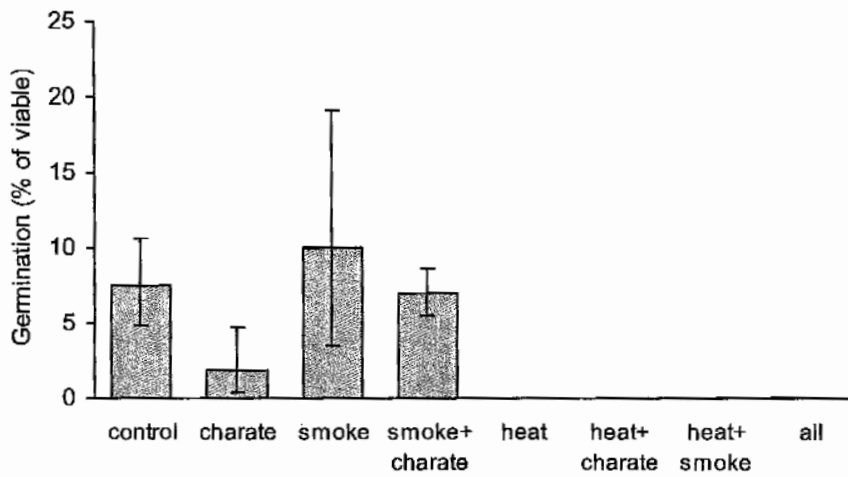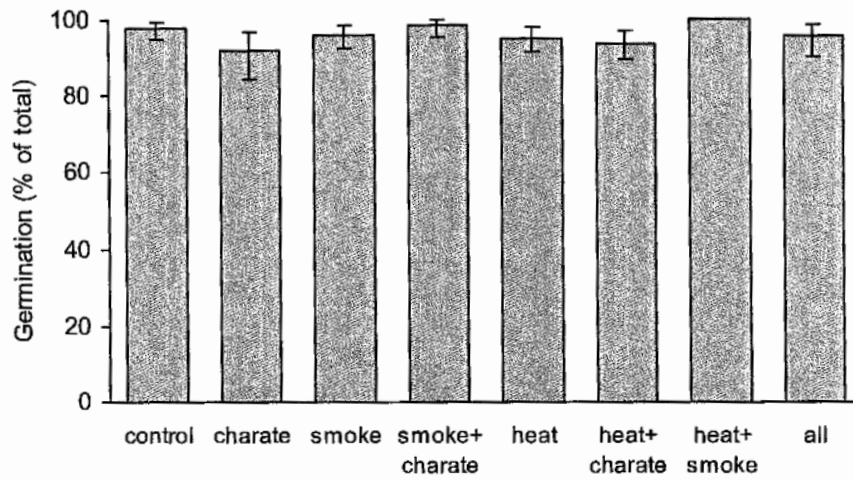
Figure 5.1 Germination responses of (a) *Haemodorum planifolium* and (b) *Lomandra longifolia* to fire-related germination cues. No treatment effects apparent except lethal heat effect in *L. Longifolia*. Back-transformed data; error bars are standard error. See Table 5.3 for ANOVA results.
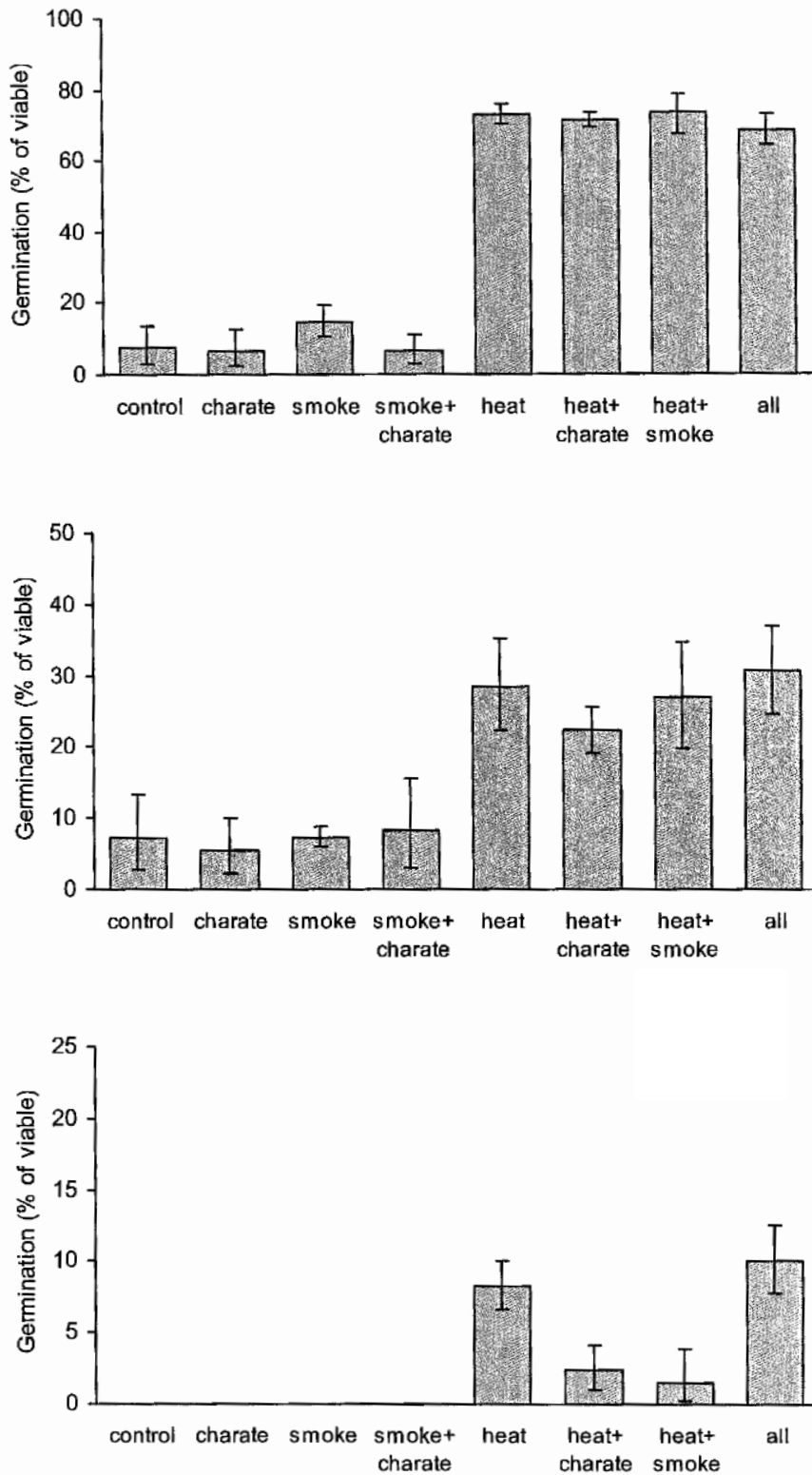
Figure 5.2 Germination responses of (a) *Acacia suaveolens*, (b) *Acacia terminalis*, and (c) *Boronia ledifolia* to fire-related germination cues. Heat effect only. Back-transformed data; error bars are standard error. See Table 5.3 for ANOVA results.
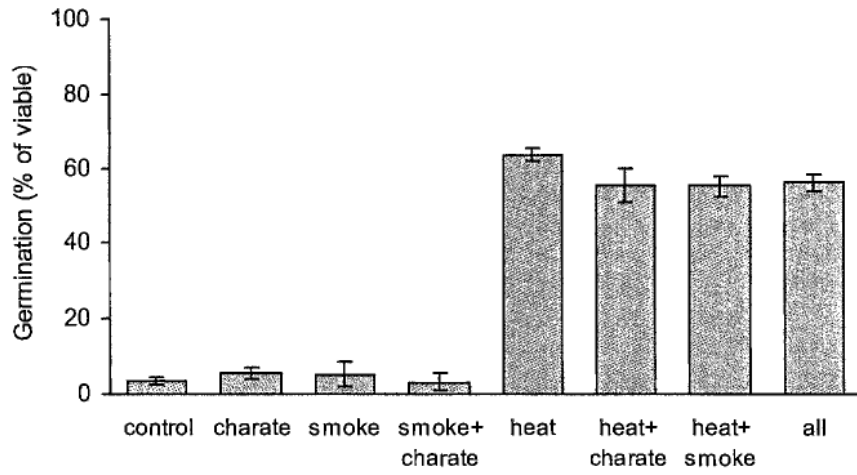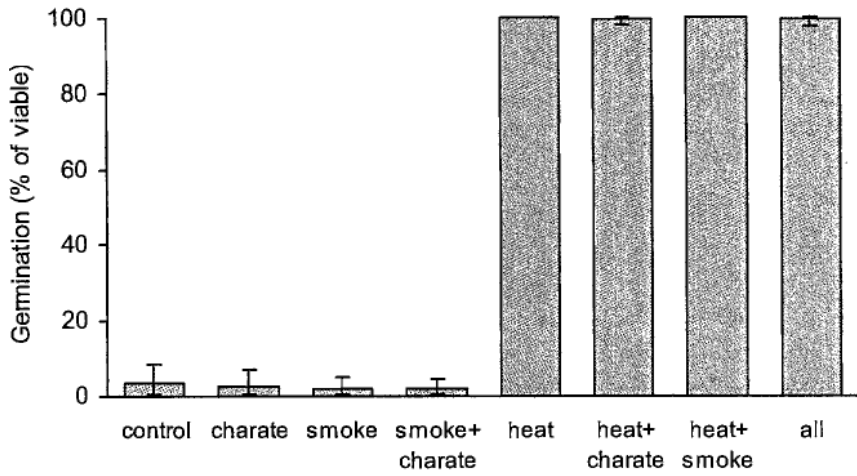
Figure 5.2 continued Germination responses of (d) *Dillwynia retorta* and (e) *Dodonaea triquetra* to fire-related germination cues. Heat effect only. Back-transformed data; error bars are standard error. See Table 5.3 for ANOVA results.
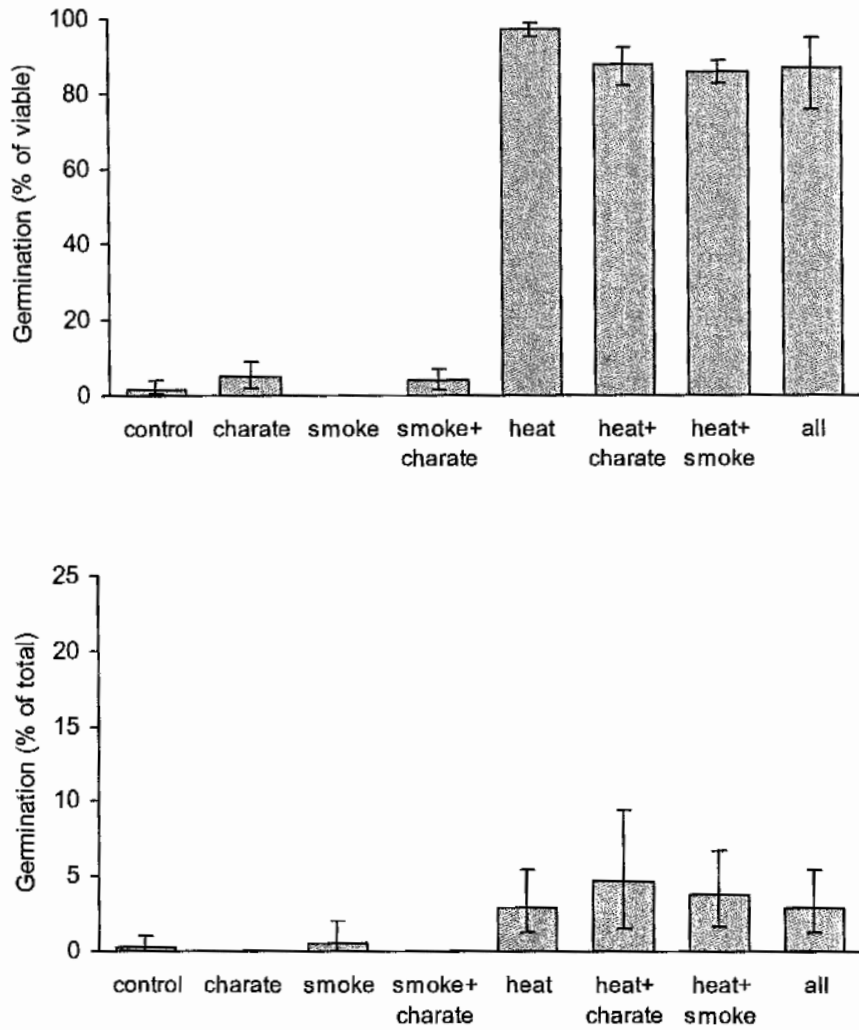
Figure 5.2 continued Germination responses of (f) *Lasiopetalum ferrugineum* and (g) *Zieria laevigata* to fire-related germination cues. Heat effect only. Back-transformed data; error bars are standard error. See Table 5.3 for ANOVA results.
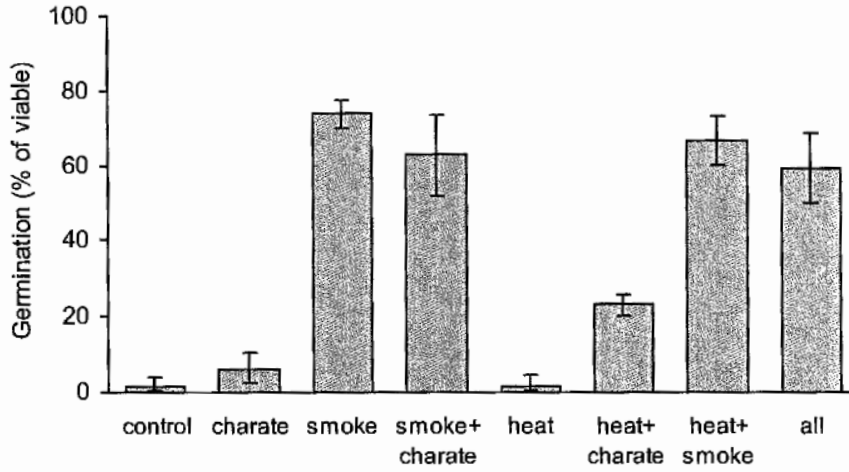
Figure 5.3 Germination response of (a) *Calytrix tetragona* to fire-related germination cues. Smoke effect only. Back-transformed data; error bars are standard error. See Table 5.3 for ANOVA results.
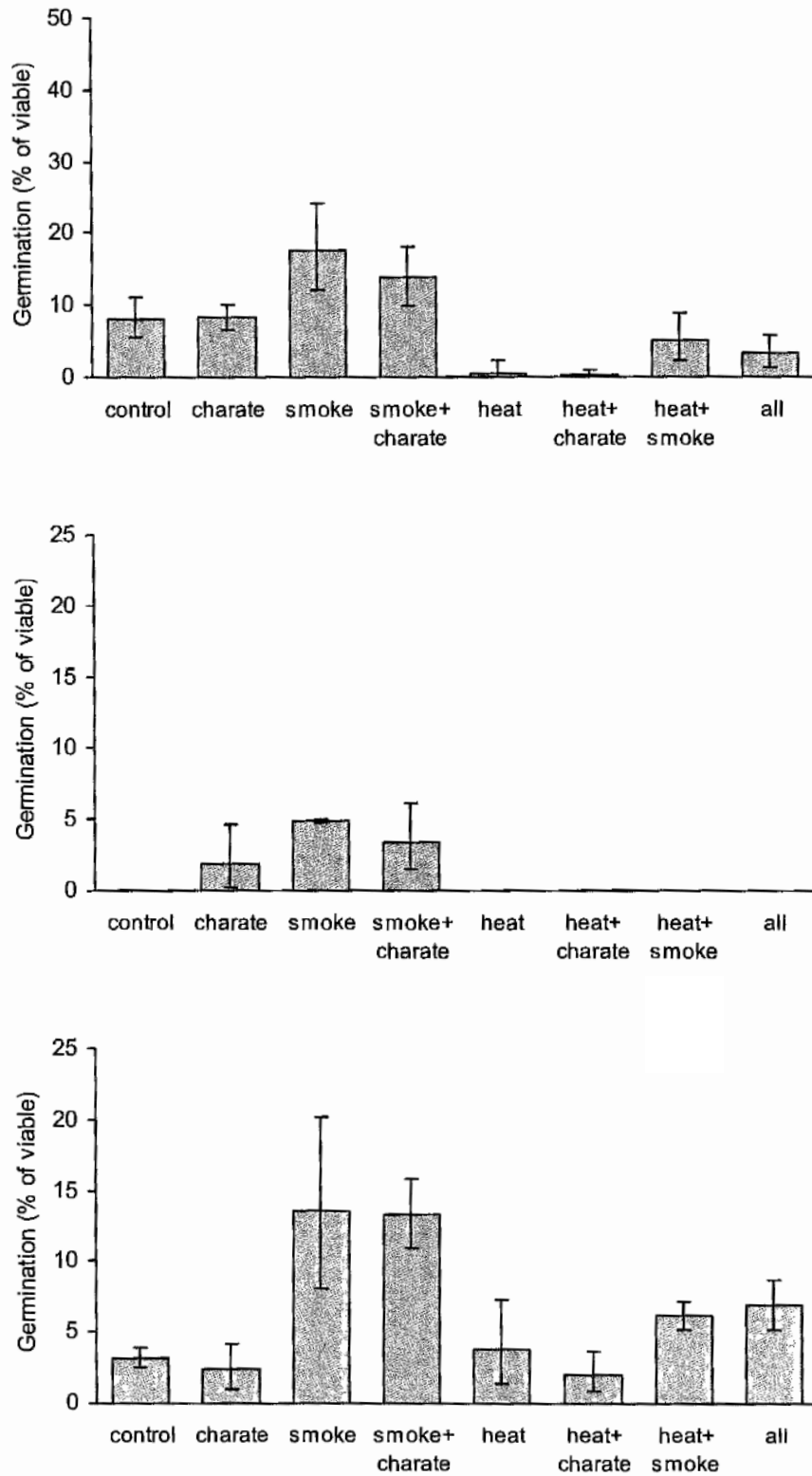
Figure 5.3 continued Germination responses of (b) *Bauera rubioides,* (c) *Pimelea linifolia* (seed lot b), and (d) *Woollsia pungens* to fire-related germination cues. Smoke effect positive, heat inhibitory or lethal. Back-transformed data; error bars are standard error. See Table 5.3 for ANOVA results.
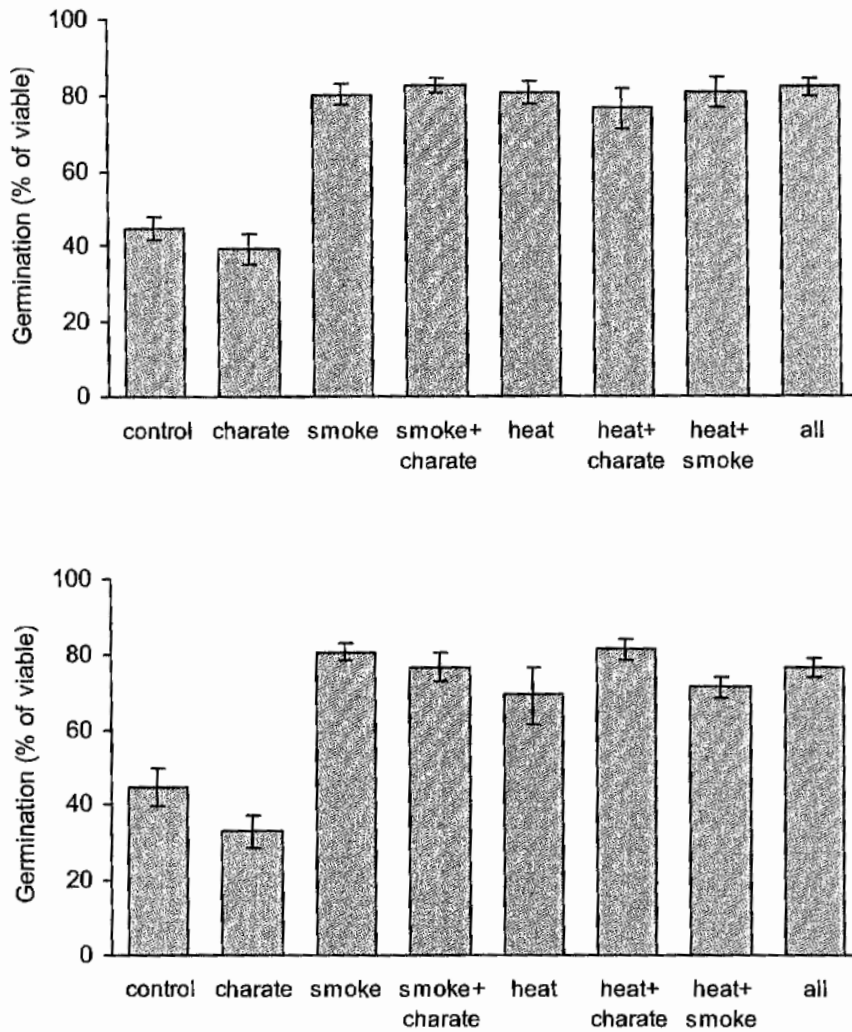
Figure 5.4 Germination responses of (a) *Epacris microphylla* (seed lot a) and (b) *Epacris microphylla* (seed lot b) to fire-related germination cues. Heat and smoke effects equal. Back-transformed data; error bars are standard error. See Table 5.3 for ANOVA results.
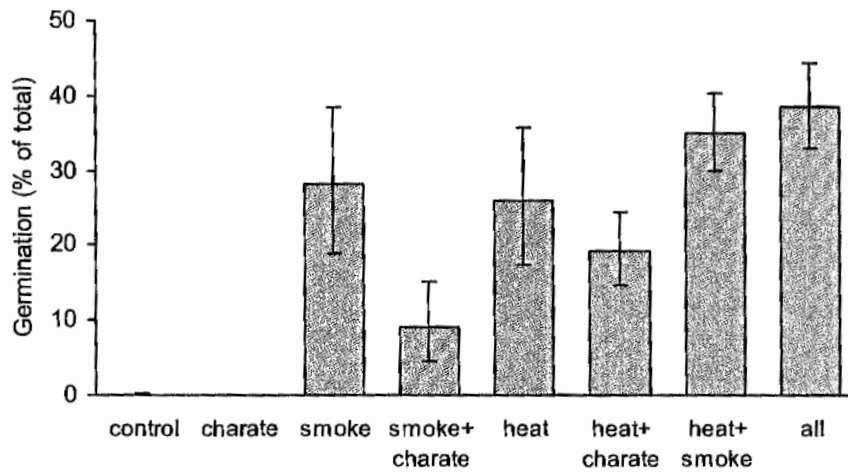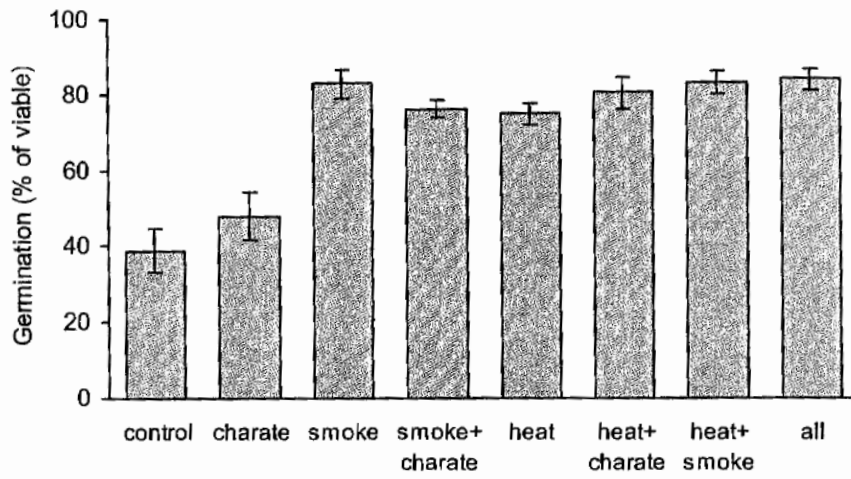
Figure 5.4 continued Germination responses of (c) *Kunzea ambigua* and (d) *Sprengelia incarnata* to fire-related germination cues. Heat and smoke effects equal. Back-transformed data; error bars are standard error. See Table 5.3 for ANOVA results.
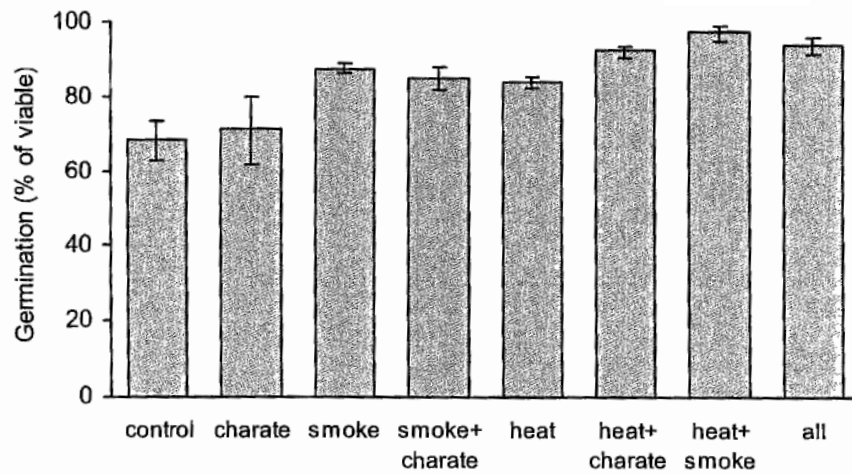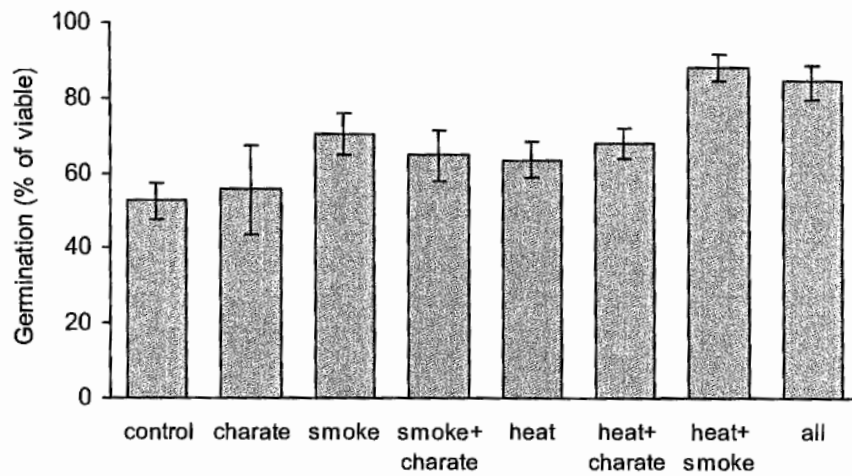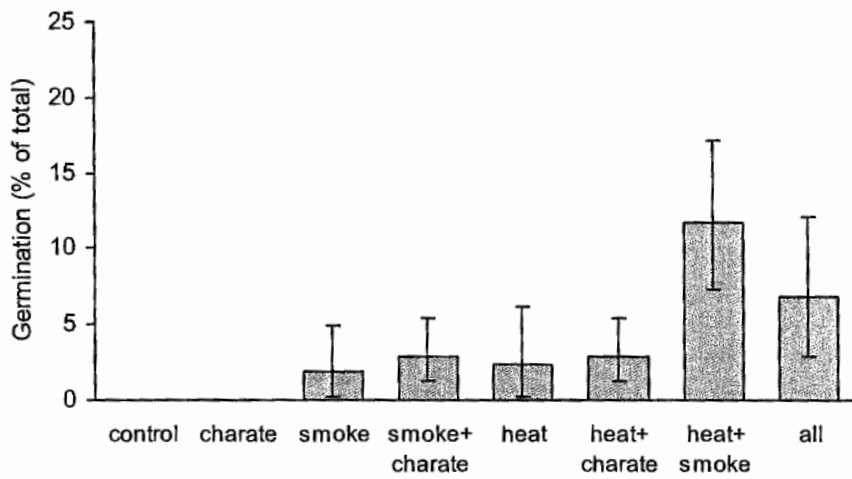
Figure 5.5 Germination responses of (a) *Gahnia sieberiana*, (b) *Grevillea sericea* (seed lot a), and (c) *Kunzea capitata* and to fire-related germination cues. Heat and smoke effects equal and additive. Back-transformed data; error bars are standard error. See Table 5.3 for ANOVA results.
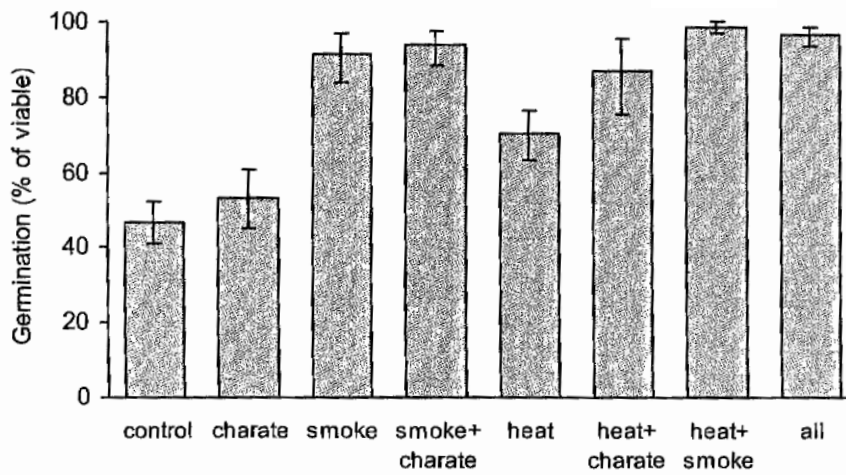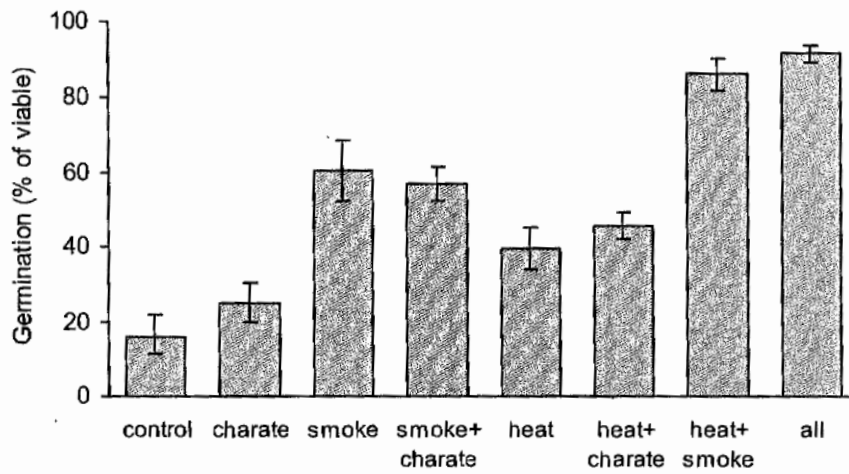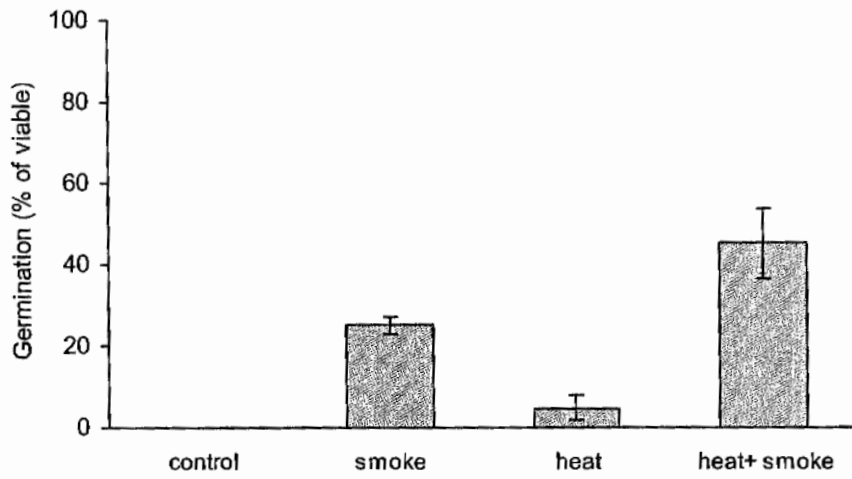
Figure 5.5 continued Germination responses of (c) *Grevillea buxifolia,* (d) *Grevillea sericea* (seed lot b), and (e) *Grevillea speciosa* and to fire-related germination cues. Heat and smoke effects unequal and additive. Back-transformed data; error bars are standard error. See Table 5.3 for ANOVA results.
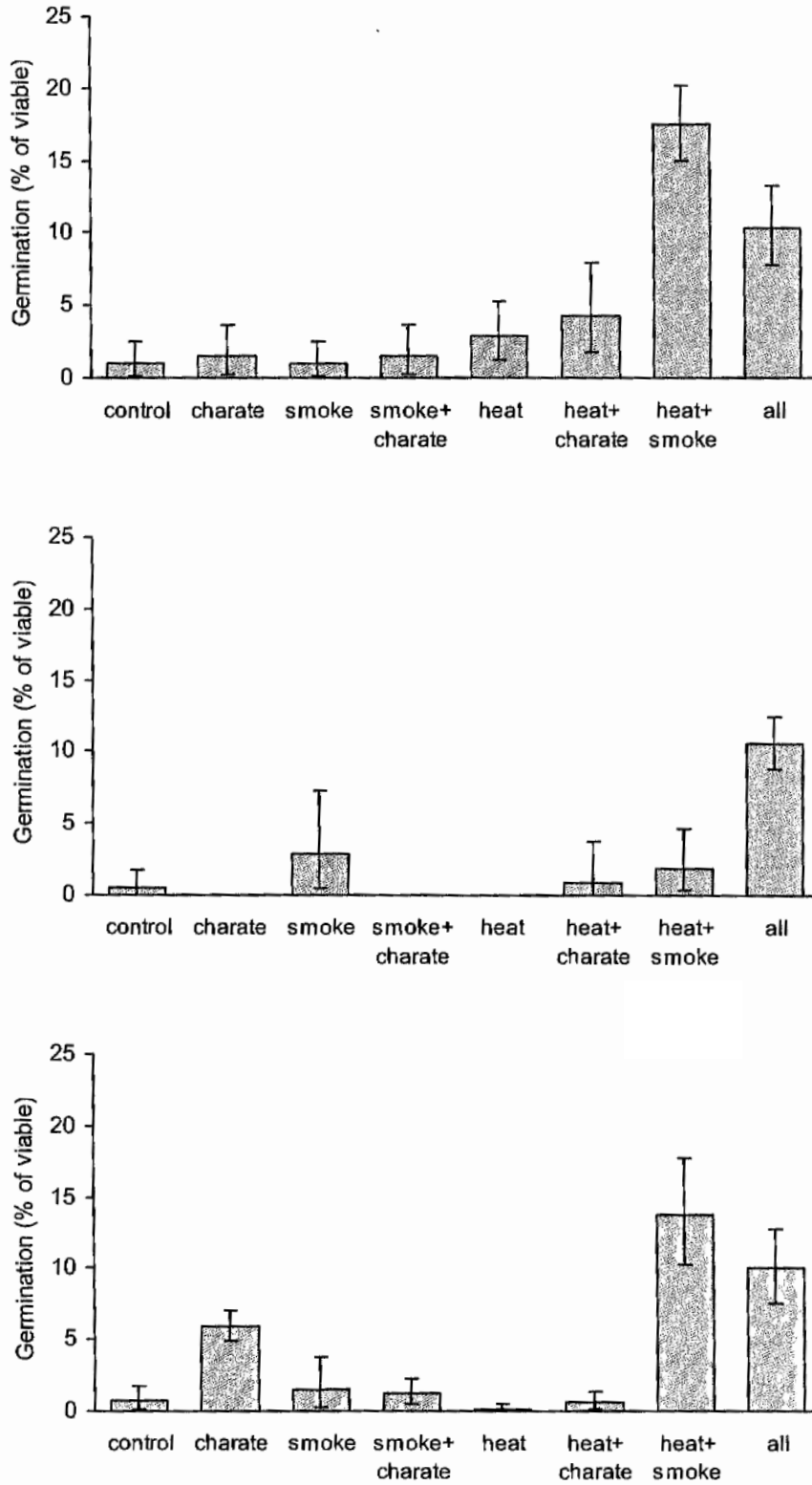
Figure 5.6 Germination responses of (a) *Conospermum taxifolium* (seed lot b), (b) *Eriostemon australasius,* and (c) *Mitrasacme polymorpha* to fire-related germination cues. Heat and smoke work in unison only. Back-transformed data; error bars are standard error. See Table 5.3 for ANOVA results.

Seed Lot Comparisons

For *Grevillea sericea,* in which seed lots were collected from different seasons of the same year, the summer seed lot had both higher viability and higher dormancy than that collected in winter. However, the treatment effects and maximum germinability of the two seed lots were similar.

For the other three species with two seed lots, the seed lots were collected from the same season of different years. Viability was not decreased during storage, and the dormancy level remained stable. For *Epacris microphylla* the two seed lots behaved the same, responding the same way to the treatment effects and achieving the same maximum germinability. In both *Conospermum taxifolium* and *Pimelea linifolia,* the older seed lot achieved only low germination levels and showed no treatment effects, while the younger seed lot did respond to the applied treatments.

Onset of Germination

For all *Grevillea* species smoke greatly reduced the time taken to commence germination (Table 5.4; Fig. 5.7). Heat also had a small influence on germination onset of *G. speciosa* (heat treatment gave a one day reduction in first germination compared to a seven day reduction with smoke treatment). For *G. buxifolia* a significant interaction between smoke and heat indicated that the smoke-induced reduction in germination onset was greater in the absence of heat. This, however, was an artefact of the failure of control seeds to germinate at all. Charred wood had no significant impact on germination time.

Functional Groups

The germination response to fire-related cues was found to be related to the defined functional groups ($P = 0.005$; Table 5.5). The majority of species showing no treatment effects are resprouters (five resprouters, three variable fire response, one seeder with wide dispersal; functional groups 2-4). Species with only a heat response are mostly seeders (five obligate seeders, two variable; functional groups 1-2). Species showing a smoke response only all have a variable fire response (functional group 2). The species with a combined heat and smoke response (either equal or additive) are generally seeders with some resprouting capacity (two obligate seeders, seven facultative seeders; functional groups 1-2), and the species only germinating with combined heat and smoke are all able to resprout (three variable, one resprouter; functional groups 2-3).

Table 5.4 Onset of germination in *Grevillea* species. Seed lot is differentiated (a, b; see Chapter 2 for details) where two seed lots were tested. Results of three-factor ANOVA; F-ratio given, significance indicated: * $0.010 < P \leq 0.050$, ** $0.001 < P \leq 0.010$, *** $P < 0.001$. † Charred wood not tested for *G. buxifolia*.

Factor	*G. buxifolia*	*G. sericea* a	*G. sericea* b	*G. speciosa*
smoke	37.203***	125.597***	34.129***	176.079***
heat	4.053	3.568	1.306	8.193**
charred wood	†	0.973	0.403	0.314
smoke×heat	5.353*	0.042	0.790	0.148
smoke×charred wood	†	0.208	0.790	0.814
heat×charred wood	†	0.154	1.032	0.058
smoke×heat×charred wood	†	2.942	1.032	1.337
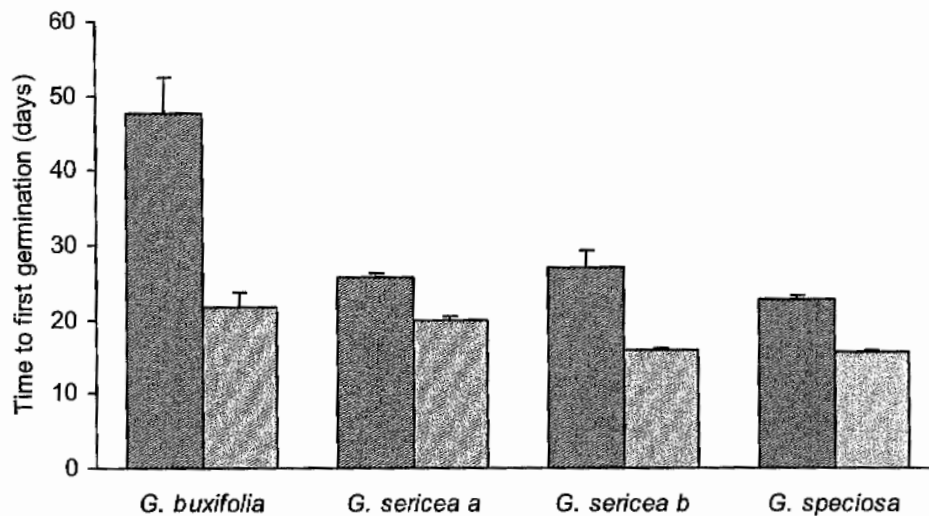
Figure 5.7 Onset of germination in *Grevillea* species. Data has been pooled into non-smoke (dark grey bars) and smoke treatments (light grey bars). Error bars are standard error. Seed lot is differentiated (a, b; see Chapter 2 for details) for *G. sericea*.

Table 5.5 Relationship between functional groups and germination response. The number of species in each group is shown; expected values based on the null hypothesis of independence are given in parentheses. Functional groups: 1 = post-fire seedling recruitment essential, 2 = recruitment important but not critical, 3 = recruitment not essential (including 4 = species without persistent seedbanks); see text for full details. Germination responses are from Table 5.3.

Germination response	Functional group			Chi-square test:
	1	2	3 and 4	$df = 6$, .05 level, R: $\chi^2 \geq 12.592$
no effect	0 (2.1)	5 (5.9)	5 (2.1)	$\chi^2 = 21.26$, $P = 0.0016$
heat only	5 (1.4)	2 (4.1)	0 (1.4)	**Monte Carlo permutation:**
smoke only	0 (0.8)	4 (2.4)	2 (0.8)	$X^2 = 19.39$, $P = 0.005$
both heat and smoke	2 (2.7)	9 (7.6)	2 (2.7)	

Discussion

Germination Response

Most of the species that were unaffected by any germination treatment either failed to germinate at all, or had very low levels (<5%) of germination across all treatments. Either the treatments used have failed to break the dormancy of these seeds, or the conditions (e.g. temperature, light and moisture levels) under which the trials were held did not meet their germination requirements. *Haemodorum planifolium*, which showed no dormancy and no treatment response, is a pyrogenic flowering species with a transient seedbank. It resprouts very quickly following fire and is stimulated to flower, releasing non-dormant seeds into the post-fire environment to germinate immediately. *Lomandra longifolia*, which displayed a lethal response to heat (also seen in the heat range trial; Chapter 4), also shows rapid post-fire flowering as well as tolerant establishment. It possibly has very low seedling establishment following fire, as many authors (e.g. Purdie & Slatyer 1976; Benwell 1998) have considered it to be an obligate resprouter.

The majority of species studied had a high level of seed dormancy (≤10% control germination). As discussed above, *H. planifolium* has a transient non-dormant seedbank. Of the other five species (*Epacris microphylla, Grevillea sericea, Grevillea speciosa, Kunzea ambigua* and *Kunzea capitata*) showing only moderate dormancy (18-68% control germination), other evidence has shown that these genera possess variable dormancy levels (Auld & Tozer 1995, Edwards & Whelan 1995, Auld *et al.* 2000). Edwards & Whelan (1995) have described *Grevillea* dormancy as polymorphic, with a single seed lot displaying innate, enforced and induced dormancy. These *Kunzea* species have been shown to possess imposed secondary dormancy, having a large non-dormant seed fraction that remains persistent under field conditions (Auld *et al.* 2000).

The general lack of a charred wood response is interesting given that many South African and Californian species respond to a similar degree when charate and smoke treatments are compared (Brown 1993a, Keeley & Bond 1997). However, Australian species have shown little charred wood response (although it has not been thoroughly investigated). Bell *et al.* (1987) found only one of 39 Western Australian species (*Burchardia umbellata*) had its germination enhanced by charred wood. Eleven of these species (including *B. umbellata*) have since been found to have a positive smoke response (Dixon *et al.* 1995, Roche *et al.* 1997). In a soil seedbank study of several Tasmanian vegetation communities, Marsden-Smedley *et al.* (1997) found that charred wood gave no increase in seedling emergence of 120 native and exotic plants, while smoke treatment did. Of 71 of these species analysed individually, only one exotic plant had a positive charred wood response (Marsden-Smedley *et al.* 1997). A lack of charred wood response has also been suggested for species of phrygana communities of the Mediterranean Basin (Keeley & Baer-Keeley 1999), though no comparison to smoke response has been made.

The species that responded to heat only all have hard seed coats. Such species are well known for their heat response in this temperature range, as the seed coat is fractured, removing the physical dormancy that it imposed (Auld & O'Connell 1991). While most of these species possess only this physical dormancy, Rutaceae seeds have both seed coat and embryo imposed dormancy, and while seedling establishment is prolific in the field after bushfire, in trials their germination has proven to be difficult and erratic (Whitehorne & McIntyre 1975, Plummer 1996). This is reflected in the low levels of germination

achieved here even in the positive heat treatments. The other two Rutaceae species studied also had very low germination, with *Phebalium squamulosum* showing no treatment effects and *Eriostemon australasius* requiring all three germination cues combined.

Relatively few species responded to smoke only, and most of these were sensitive to heat. While heat had no influence on *Calytrix tetragona*, it was inhibitory or lethal to *Bauera rubioides*, *Pimelea linifolia* and *Woollsia pungens*, counteracting the positive influence of smoke. Similar responses were seen in the heat range trials, with inhibitory or lethal temperatures of 90 or 100 °C seen for these latter three species but *C. tetragona* unaffected even at 120 °C (Chapter 4). Similarly, Grant & Koch (1997) found 11 out of 13 smoke responding species were inhibited by high temperatures.

That heat can counteract the smoke response leaves these seeds quite vulnerable, they need to be protected from inhibitory temperatures and yet still receive a smoke stimulus. Receiving the smoke stimulus may not be a limiting factor as smoke may penetrate through soil to depths of 8 cm (Chapter 6). However, whether seedlings can emerge from depths at which the soil temperature is not too high is questionable. Inhibitory temperatures (80 °C) may be experienced in the top 2 cm of soil under a moderate intensity fire (Bradstock & Auld 1995). The maximum depth from which a seedling can emerge has been shown to be inversely related to seed size (Bond *et al.* 1999). The seeds of these three species are all quite small (*Bauera rubioides* 0.32 mg, *Pimelea linifolia* 1.6 mg, *Woollsia pungens* 0.2 mg; Table 2.3), and using the equation developed by Bond *et al.* (1999) (developed from fynbos species and conditions) would only emerge from depths of 2-3 cm. It is thus predicted that these species are more likely to have their seeds survive, germinate and emerge from fires of reasonably low intensity. Species with heat sensitive seeds have been shown to germinate only or more prolifically after lower intensity fires (Moreno & Oechel 1991, Tyler 1995) but no indication of smoke response was given. The opposite relationship has been shown for heat-stimulated species, with legumes germinating far more prolifically after high intensity fires (Christensen & Kimber 1975, Auld 1986b, Auld & O'Connell 1991).

Onset of Germination
The enhanced rate of germination seen in the *Grevillea* species tested with smoke treatment has been reported before (Brown 1993b, Roche *et al.* 1997a, Morris 2000, Read *et al.* 2000). This rapid germination may give smoke cued seedlings a competitive advantage over those that are slower to emerge (Brown 1993b), and may be greatly advantageous in site rehabilitation work (Read *et al.* 2000). For a species with multiple cues, this variation in germination rate may allow emergence to be spread over a greater period of time, providing the population with a greater chance of seedling survival in areas or seasons with unpredictable rainfall.

Multiple Germination Cues
Little research to date has examined the combined effects of heat and combustion products, and while there are a few examples of the types of interactions seen here, this variety of interactive responses has not yet been shown within a single study.

The additive effects of either smoke and heat or charate and heat have been found in other species: equal and additive (*Eriodictyon crassifolium*, California; Keeley 1987; *Epacris stuartii*, Tasmania; Keith 1997), unequal and additive (various *Grevillea* species, Sydney; Morris 2000) and unequal and synergistic

(*Epacris tasmanica*, Tasmania; Gilmour *et al.* 2000; *Phacelia cicutaria*, California; Keeley *et al.* 1985). The equal response to either or both cues has also been seen elsewhere (*Rhus trilobata*, California; Keeley 1987), but to my knowledge the requirement for both cues in unison has not been previously shown.

Those species in which the two cues were equal had high levels of control germination (>38%) and high germination after treatment (>75%), while those requiring the cues combined had very low control germination (<2%) and only moderate maximum germination (<18%). The species with an additive response varied between these two extremes, with control germination between 0 and 53% and maximum germination ranging from 12 to 98%. Perhaps the increasing dependence on the presence of both cues reflects the seed dormancy level or fastidiousness in germination requirements.

Multiple germination cues in general are not uncommon (Bradbeer 1988). However, the action of multiple fire-related cues is yet to be examined in detail. Given that the different cues (heat and smoke or charred wood) signal the same event, what is the purpose of one species responding to both? Logically, if the germination response to the two cues is equal the mechanism of their action on the seed is the same, whereas an unequal or additive response indicates that there are either two mechanisms involved (i.e. multiple dormancy within the seed) or two methods for achieving the same result (i.e. different ways to affect the same type of dormancy). Unfortunately, the action of smoke in breaking seed dormancy remains unclear, and the action of heat on non-hard seeds has received little attention.

Because smoke has been shown to influence the germination of such a wide range of species, it is conceded that the mechanism of smoke action probably differs between species (Keeley & Fotheringham 1998a), and it seems apparent that smoke may be able to act on both physical and physiological dormancy. Most work has assumed that smoke is acting on an embryo dormancy mechanism by initiating metabolic activity (Baldwin *et al.* 1994), increasing hormone sensitivity (van Staden *et al.* 1995b), enhancing hormone activity (Thomas & van Staden 1995), or inactivating a germination inhibitor (Keeley & Nitzberg 1984, Pierce *et al.* 1995). Alternatively, smoke may be acting on physical dormancy, perhaps through chemical scarification of the seed coat (Egerton-Warburton 1998, Keeley & Fotheringham 1998b).

The species that responded to both heat and smoke were mostly not hard seeded. However, water impermeability of the seed coat is not the only form of physical dormancy. The seed coat can also impose dormancy through restriction of oxygen diffusion, mechanical restriction of the radicle, preventing the exit of chemical inhibitors from the embryo, or containing chemical inhibitors itself (Bradbeer 1988). Hence heat can still have a positive effect through scarification to allow the movement of oxygen, exit of inhibitors and growth of the radicle, and through thermal breakdown of inhibitors (Bell *et al.* 1993, Brits *et al.* 1999).

Morris *et al.* (2000) have recently investigated the dormancy mechanisms of two *Grevillea* species, which have water permeable but dormant seeds. Both species were found to have a seed coat imposed dormancy that was not related to inhibitors within the seed coat itself. *G. linearifolia*, which responds equally to heat, smoke and the two combined, had seed coat dormancy only. In addition to this seed coat dormancy *G. wilsonii* also had an embryo imposed dormancy which was overcome by smoke.

It remains unclear whether the response to both cues is occurring at the level of individual seeds or within a seed lot. It is likely that a seed with only physical dormancy can respond to either cue, whereas a seed with both physical and embryo dormancy might require both. However, many species have polymorphic dormancy, where individual seeds within a seed lot are in different dormancy states (Keeley 1991), thus some seeds may be responding to heat, some to smoke, and some to the combination.

Establishment

A species that can respond equally to either cue would be able to establish under a wider range of fire conditions than a species with only one cue. Hard-seeded species will only germinate when exposed to sufficient heat, and are therefore disadvantaged by low intensity fire, while species with only a smoke cue and sensitivity to heat will be disadvantaged by high intensity fire. If either cue will give the same result, smoke will allow germination when soil heating is inadequate, and vice versa.

Where there is an additive relationship between the two cues, the presence of both would be required for maximum seedbank response. So, while some seeds will still germinate when soil heating is inadequate for a heat response, some seeds will remain unaffected. This makes these species less fastidious of fire conditions than species with one cue, but more fastidious than those with equal response to the cues.

Most fastidious of all are the species which require both cues in unison for any germination to occur. These species are likely to encounter many fires unsuitable for their establishment. By limiting recruitment opportunities to the occasions when the two stimuli coincide adequately, population viability may be threatened (Keith 1997).

Functional Groups

A relationship was found between germination effect of the cues and the functional groups (fire responses) of the species. The species have been divided into groups that reflect their level of dependence on recruiting seedlings after fire. Obligate seeders (functional group 1) are entirely reliant on post-fire seedling recruitment as all adults are killed. This needs to be through germination of the on-site soil seedbank, as dispersal is generally poor in species with a persistent soil seedbank (Keeley 1991), limiting any opportunity for re-invasion of an area. If establishment is poor then the population is reliant on the remaining seedbank to persist through to the next fire event (Keith 1996). Facultative seeders (functional group 2) are highly reliant on seedling recruitment, but show a limited capacity to resprout (though this may depend on variables such as fire intensity and plant health). This limited resprouting capacity gives the population a better chance of persistence in the event that establishment of seedlings is poor. Resprouters (functional group 3) respond to fire mainly by vegetative regeneration of existing adult plants. They are not dependent on seedling recruitment after each fire, but still take advantage of the improved establishment conditions of the post-fire environment to replenish their population, as some fire mortality and natural senescence occurs (Auld & O'Connell 1991). They generally have less prolific post-fire recruitment than do seeders (Keeley & Zedler 1978, Moreno & Oechel 1992, Benwell 1998). Traits such as wide seed dispersal, rapid post-fire flowering and tolerant establishment reduce the reliance on germination of the on-site seedbank for all these groups (Noble & Slatyer 1980). Resprouters with transient seedbanks and rapid post-fire flowering (functional group 4) were included in this study to allow comparison with seeds that are not expected to be adapted to any fire cue (Keeley & Bond 1997). In

previous experiments three such species (*Doryanthes excelsa*, *Haemodorum planifolium* and *Xanthorrhoea resinifera*) were found to have a negative response to heat-shock treatment (Chapter 4).

Of the species which showed no response to the fire-related cues most were resprouters, including those with transient seedbanks. *Pimelea linifolia* (a facultative seeder) had one seed lot with no treatment effects, but had the younger seed lot respond to smoke. The two obligate seeders that didn't respond (*Cassytha pubescens* and *Persoonia pinifolia*) have fruit adapted for wide dispersal by birds or mammals and can thus re-invade a site from other populations. *Persoonia pinifolia* has proven elsewhere to be extremely difficult to germinate (Whitehorne & McIntyre 1975), and may have germination requirements that were not explored in this study. For example, extremely recalcitrant species might require soil storage for a period before a fire stimulus is successful (Keeley & Fotheringham 1998a).

The species with the most fastidious requirements for fire-related cues (those requiring heat and smoke in unison) all have resprouting capacity. So while their strict germination requirements may limit their post-fire establishment opportunities, this is not of great concern to population persistence. Two of these species further ensure their population survival through the persistence or replacement of their seedbanks. *Conospermum taxifolium* has been shown to have a very persistent seedbanks (Auld *et al.* 2000), while *Mitrasacme polymorpha* is a facultative pyrogenic flowerers, taking the opportunity to replenish the seedbank after a fire. *Eriostemon australasius* has been shown to recruit prolifically after fire but is extremely difficult to germinate in experiments (Whitehorne & McIntyre 1975), and so may require something not provided in these experiments.

The species with heat and smoke effects either equal or additive were seeders, mostly with limited resprouting capacity. These species are predicted to establish under the widest variety of fire conditions. Interestingly, most of these species have a moderate or high proportion of non-dormant seeds, and so perhaps have a less persistent seedbank than do species with high innate dormancy (Keeley 1991). This would make establishment after every fire event important for population persistence.

The species with only a smoke response all have a variable fire response. Three of these species have seeds that are sensitive to heat, and as discussed above are predicted to establish only after lower intensity fires. They are also only likely to resprout after a low intensity fire, and so are predicted to decline under a regime of high intensity fires. This has not yet been examined through population studies. *Bauera rubioides* tends to be found in wet, shaded habitats (Harden 1990), which may create the low-heat, high-smoke fire conditions that would favour germination.

The heat responders were mostly obligate seeders, all with hard seed coats. While as a group these are the most reliant on post-fire establishment, having only one cue limits the fire conditions under which their germination needs will be met. However, the hard seed coats which give them this need for high temperatures also bestow a high degree of seed longevity and seedbank persistence (Auld 1986b). A single low intensity fire will result in poor recruitment (Auld & O'Connell 1991), but the population may still recover after the next fire event. However, under a regime of low intensity fires the species is at danger of extinction (Auld & O'Connell 1991).

While the role of fire frequency has been well studied in relation to its effect on plant community dynamics (e.g. Morrison *et al.* 1995, Bradstock *et al.* 1997), it is apparent that fire intensity also has a large influence through its effect on germination. The role of fire intensity in the populations of leguminous species has been shown, but the interactions between fire intensity and species with smoke and combined heat and smoke responses needs to be evaluated.

CHAPTER 6: GERMINATION CUE EFFECTS ON BURIED SEEDS

Aim

Since its discovery as a germination cue (de Lange & Boucher 1990), most experiments on smoke have been concerned with either finding a species' optimal germination cue (e.g. Brown *et al.* 1993, Dixon *et al.* 1995) or looking for the active ingredients of the smoke cue (e.g. van Staden *et al.* 1995a). Very little has been done to investigate how the smoke cue is received by buried seeds or the effectiveness of natural fires in providing the smoke cue. Variations in fire intensity and duration, as well as environmental factors such as post-fire rainfall, soil type and soil moisture may affect the way in which seeds receive fire-related germination cues. Given all these variables, germination responses in the field may vary markedly from those seen under controlled laboratory conditions. It is therefore hard to predict the proportion of the seedbank likely to germinate after a fire, and hence population dynamics with different fire regimes.

This chapter investigates the ability of smoke to reach buried seeds, as well as examining the performance of the fire-related cues previously studied in the laboratory (Chapters 3-5) when applied under field conditions and in comparison to a prescribed fire.

Two trials were performed to investigate parameters that may influence the ability of a smoke cue to reach buried seeds. The first experiment looks at both the time taken for smoke to reach buried seeds and whether water is a necessary agent of smoke transport. The second experiment investigates the depth to which smoke may penetrate. These experiments were performed on *Grevillea* seeds, as these were know to have smoke-enhanced germination (Chapter 5), a large quantity of seed was available, and seeds were large enough to be easily retrieved from soil samples.

Another experiment investigated the effect of a fire and individual fire-related cues on seeds buried under field conditions. Species choice was based on several factors: availability of seed; large seeds were required for logistic reasons; and known response to germination cues from previous experiments. The four species used were: *Acacia suaveolens*, a known heat-responder; two *Grevillea* species, known to respond to both heat and smoke; and *Persoonia pinifolia*, which failed to germinate in previous experiments (Chapter 5).

Methods

Smoke Effect on Buried Seeds

(A) Time And Water Effects

Grevillea sericea seeds (seed lot c; 25 seeds per replicate; seed age 19 months; viability 91.2 ± 1.1%) were buried in small aluminium trays (90 cm^2, 2 cm deep). Seeds were placed on top of 0.5 cm depth of washed river sand, with a further 1.5 cm of sand packed gently on top of the seeds. Twenty four trays received 20 minutes of aerosol smoke (smoking methods as per Chapters 3 and 5); with four replicate trays per treatment being smoke-treated separately. Twelve of these trays were watered following smoke treatment with 40 ml of distilled water applied as a mist spray. Eight control trays received no smoke treatment, and half of these were watered. All trays were then sealed in individual plastic bags and left for

a period of one, two or four weeks, after which seeds were removed from the sand and placed in Petri dishes (see below). There were also two unburied seed controls, one untreated and one smoke-treated (20 minutes of aerosol smoke as above). See Table 6.1 for a summary of the treatments applied.

(B) Depth Of Burial Effects

Grevillea speciosa seeds (seed lot c; 25 seeds per replicate; seed age 9 months; viability $97.0 \pm 0.7\%$) were buried in plastic pots (9 cm diameter, 9 cm deep). Seeds were placed on top of at least 1 cm depth of washed river sand, with various amounts (2, 4, 6, 8 cm) of sand packed gently above the seeds. All pots received 20 minutes of aerosol smoke; with four replicate pots per treatment being smoke-treated separately. Following smoke treatment all pots were watered with 50 ml of distilled water applied as a mist spray. Pots were then sealed with plastic film and left for one week, after which seeds were removed from the sand and placed in Petri dishes. There were also two unburied seed controls, one untreated and one smoke-treated (20 minutes of aerosol smoke as above), but no control buried seeds due to lack of sufficient seed. See Table 6.2 for a summary of the treatments applied.

Table 6.1 Treatments applied to *Grevillea sericea* in experiment 1a (effects of time and water on smoke penetration).

Seeds treated	Smoke applied	Sand watered	Time left buried
loose	no	n/a	n/a
loose	yes	n/a	n/a
buried	no	no	4 weeks
buried	no	yes	4 weeks
buried	yes	no	1 week
buried	yes	no	2 weeks
buried	yes	no	4 weeks
buried	yes	yes	1 week
buried	yes	yes	2 weeks
buried	yes	yes	4 weeks

Table 6.2 Treatments applied to *Grevillea speciosa* in experiment 1b (effects of burial depth on smoke penetration). Buried treatments were all watered and left buried for 1 week.

Seeds treated	Smoke applied	Burial depth
loose	no	n/a
loose	yes	n/a
buried	yes	2 cm
buried	yes	4 cm
buried	yes	6 cm
buried	yes	8 cm

General

Following treatment seeds of both species were placed in Petri dishes lined with Whatman No. 1 filter paper and watered with distilled water. Dishes were re-watered as required to retain moisture, and periodically checked for germination. Germination was determined as being when the radicle emerged, and germinated seeds were removed from the Petri dish. Dishes were kept under ambient laboratory conditions in a dark cabinet (but checked in the light). Trials ran until no further germination was recorded for at least one week. At the end of the trial viability of the remaining seeds was assessed via a

cut test. Each seed was cut open to expose the embryo, and graded as viable (healthy, plump, white embryo), dead or empty. Germination was expressed as a percentage of the number of viable seeds available per replicate (germinated plus viable remaining seed).

Treatment effects were assessed by one-way analysis of variance (ANOVA) and post hoc Tukey honestly significant difference (HSD) multiple comparison. Data were assessed for homogeneity of variance via Cochran's test prior to ANOVA. For both species treatments were pooled into buried and unburied to test for differences in seed viability due to seed burial. For *G. sericea*, burial time effects on germination were tested for separately for both the watered and unwatered smoke-treated, buried seeds. The three burial times were then pooled into watered and unwatered for further analysis. Cochran's test was unsatisfactory on both raw and arcsine-transformed germination data for the remaining six treatments applied to *G. sericea*. As the large differences in variance were discreet between control and smoke treatments (see Fig. 6.1); control and smoke treatments were analysed separately to test for differences between unburied, buried and unwatered, and buried and watered seeds. Finally, treatments were pooled into control and smoke-treated to test for smoke effects, however Cochran's test could not be satisfied. For *G. speciosa*, a single ANOVA and Tukey test were performed on germination data.

Fire and Fire-cue Effects on Buried Seeds

The effects on germination of fire and fire-cue treatments were tested on seeds of *Acacia suaveolens, Grevillea sericea, Grevillea speciosa* and *Persoonia pinifolia*. Treatments for this experiment were applied in the field in open woodland vegetation within Heathcote National Park, south of Sydney. A prescribed fire was performed at the site by the NSW National Parks and Wildlife Service on August 4th 1999.

Plots (each 0.25 m^2) were set up in the area to be burnt and an adjacent area to be left unburnt, with five replicate plots per treatment. At each plot seeds of the four species (Table 6.3) were buried one day prior to treatment. For each species, seeds were buried in aluminium foil trays using soil from the plot site. Seeds were buried approximately 1 cm deep within the trays, and the trays were buried approximately 2 cm deep in the soil. The trays were then removed from the plots six days after treatment.

A soil sample (c. 100 g) was collected before the fire from each plot in the area to be burnt. Each sample was weighed before and after being oven dried (80 °C for four days) to estimate pre-fire soil moisture.

Heat, smoke and charate treatments were applied in orthogonal combinations to plots within the unburnt area. All plots were cleared of surface litter material prior to treatment to minimise the confounding effect of creating smoke and charate by heating organic matter. Heat was applied by heating the soil surface with a propane torch. The temperature reached was periodically checked by inserting a thermometer into the soil to a depth of 1 cm. Heating was continued until a temperature of at least 60 °C was reached (approximately 5 minutes). Temperatures achieved at 1 cm depth ranged from 57 °C to 94 °C.

A 'smoke tent' was constructed form lined thick calico material, to cover a surface area of 0.25 m^2. The 'smoke tent' was secured with tent pegs over the plot, and smoke channelled into this from a beekeeper's burner in which leaf litter from the site was burnt. Aerosol smoke was applied to each smoke-treated plot

for 15 minutes. Charate was collected from the ground in the adjacent burnt site and scattered over the soil surface of the plots (the amount of charate collected was of an equivalent area to the plots).

Two unburied seed controls were included to test for effects of burial on seed germination and viability; one untreated and one where seeds were scarified by hand. All of the treatments applied are summarised in Table 6.4.

Table 6.3 Species used in experiment 2 (effects of fire and fire-cues on buried seeds). Seed lot is as defined in Chapter 2. Seed age when tested is given in months. Seed viability is given as mean ± standard error.

Species	Seed lot	Seeds per replicate	Seed age	Viability
Acacia suaveolens	b	20	10	86.8 ± 1.1
Grevillea sericea	c	25	8	97.9 ± 0.3
Grevillea speciosa	b	20	8	96.6 ± 0.5
Persoonia pinifolia		30	24	not measured

Table 6.4 Treatments applied in experiment 2 (effects of fire and fire-cues on buried seeds).

Seeds treated	Area buried	Treatments applied
loose	n/a	none
loose	n/a	seeds scarified
buried	unburnt	none
buried	unburnt	charate
buried	unburnt	smoke
buried	unburnt	smoke and charate
buried	unburnt	heat
buried	unburnt	heat and charate
buried	unburnt	heat and smoke
buried	unburnt	heat, smoke and charate
buried	burnt	none

After the trays were recovered from the field (6 days following treatment), seeds of *Acacia suaveolens*, *Grevillea sericea* and *Grevillea speciosa* were removed from the trays and placed in Petri dishes lined with Whatman No. 1 filter paper and watered with distilled water. Dishes were re-watered as required to retain moisture, and periodically checked for germination. Germination was determined as being when the radicle emerged, and germinated seeds were removed from the Petri dish. Dishes were kept under ambient laboratory conditions in a dark cabinet (but checked in the light). Trials ran until no further germination was recorded for at least 1 week. At the end of the trial viability of the remaining seeds was assessed via a cut test. Each seed was cut open to expose the embryo, and graded as viable (healthy, plump, white embryo), dead or empty. Germination was expressed as a percentage of the number of viable seeds available per replicate (germinated plus viable remaining seed).

Persoonia seeds can be very slow to germinate (Chapter 5; Roche *et al.* 1997a) and may require a period of soil burial before germination (Roche *et al.* 1997a). Hence seeds of *Persoonia pinifolia* were left buried in the trays and placed in a temperature-controlled glasshouse with daily watering for 6 months. The trays were checked periodically for seedling emergence, and germination expressed as a percentage of total seed available.

The prescribed fire was performed under mild conditions which hampered both ignition and flame spread. The resulting fire was very patchy in its spatial distribution, with wide variation in the levels of vegetation scorch and litter consumption achieved. Hence, following the fire the plots in the burnt area were examined to determine the degree to which they were actually burnt. They were assessed by the attributes: scorching of vegetation immediately surrounding the plot; consumption of litter immediately on and surrounding the plot; and ground coverage of post-fire charate on the plot. Only two of the five plots were classified as fully burnt (surrounding vegetation scorched, litter consumed both on and around the plot, charate coverage of >75% on the plot), one plot was classed as partially burnt (surrounding vegetation scorched, litter partially consumed on and around the plot, charate coverage of 50-75% on the plot), and two were regarded as unburnt (surrounding vegetation scorched, litter on plot unconsumed, litter surrounding plot partially consumed, charate coverage <25% on plot). The unburnt plots were discarded from the results prior to analysis, leaving the burnt treatment with only three replicates.

Treatment effects were analysed by ANOVA after being assessed for homogeneity of variance via Cochran's test. Germination data were arcsine transformed to improve homogeneity of variance. Back-transformed means are presented in the graphs. Treatments were pooled into buried and unburied to test for differences in seed viability due to seed burial. Seed viability was also assessed between the individual treatments. Effects on germination of the treatments applied in the unburnt area were assessed by a three-factor orthogonal ANOVA. The control and heat treatments applied in the unburnt area were then assessed against the other treatments (burnt, unburied control, and unburied scarified) by a one-way ANOVA and Tukey test.

Results

Smoke Effect on Buried Seeds

The viability of *Grevillea sericea* seeds was not affected by burial ($P = 0.720$). Germination was enhanced by smoke treatment (Fig. 6.1), for both smoke application to loose seeds and buried seeds. There was no significant difference in germination level for seeds left buried for different times after smoke treatment, for either non-watered ($P = 0.892$) or watered ($P = 0.223$) sand. For further analysis and presentation the three burial times were pooled per watering treatment.

When seeds were buried in sand, watering of the sand improved the smoke effect on germination. Germination achieved by seeds buried in watered smoked sand was equivalent to that of loose smoke-treated seeds. Burial and watering regime had no significant effect on the germination of control seeds (Fig. 6.1).

The parameters to be used in the second experiment (*Grevillea speciosa*, depth of burial) were determined from the first experiment: that is, seeds were left buried for 1 week (as no significant time effect was seen) and sand was watered following smoke treatment (as watering improved germination).

The viability of *Grevillea speciosa* seeds was not affected by burial ($P = 0.151$). Germination was enhanced by smoke treatment, with the germination achieved by seeds buried in smoked sand being

equivalent to that of loose smoke-treated seeds. There was no significant difference between the four burial depths (Fig. 6.2).

Fire and Fire-cue Effects on Buried Seeds

Pre-fire soil moisture was variable with a mean of $15.3 \pm 7.9\%$. There was no apparent relationship between soil moisture and the patchiness of the burn: the 2 fully burnt plots had soil moisture of 7.5 and 16.0%; the partially burnt plot 15.7%; and the unburnt plots 7.9 and 19.6%.

Few seeds of *Persoonia pinifolia* germinated (mean germination $0.4 \pm 0.3\%$), with no treatment effects.

Seed viability was not different between unburied and buried seeds (*Acacia suaveolens* $P = 0.094$; *Grevillea sericea* $P = 0.446$; *Grevillea speciosa* $P = 0.381$), nor between treatments applied (*Acacia suaveolens* $P = 0.368$; *Grevillea sericea* $P = 1.000$; *Grevillea speciosa* $P = 0.800$), thus no treatment caused seed mortality.

Three-factor ANOVA on the seeds buried in the unburnt area found heat to be the only significant factor for all three species (P value for heat: *Acacia suaveolens* $P < 0.001$; *Grevillea sericea* $P = 0.008$; *Grevillea speciosa* $P = 0.015$). There were no significant interactions between the factors.

As heat was the only significant applied treatment for all species, further analysis (comparison to the burnt plots and unburied seeds) was conducted with just the control and heat treatments from the unburnt buried seeds.

Scarification was the best treatment for *Acacia suaveolens* seeds. Soil heating significantly enhanced germination, while the effects of the prescribed fire were intermediate between the control and heat treatments (Fig. 6.3a).

The two *Grevillea* species behaved in a similar way: the prescribed fire significantly improved germination compared to the control, while the effects of the heat treatment were intermediate between the fire and the control. For *G. sericea*, scarification was equivalent to soil heating, while for *G. speciosa* scarification had no effect (Fig. 6.3c).
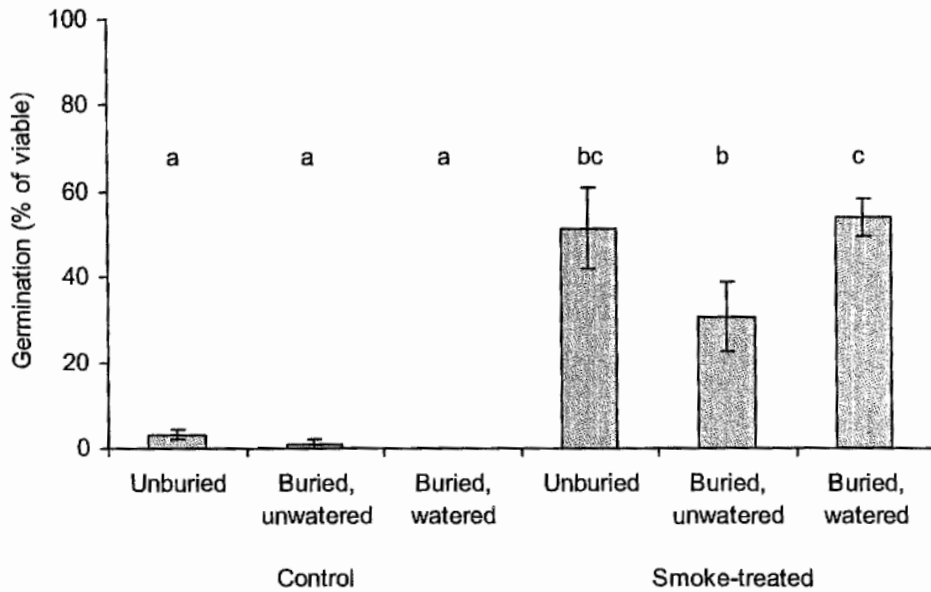
Figure 6.1 Germination response of *Grevillea sericea* to smoke treatment. Error bars are standard error. NB Data has been pooled from the three burial-time treatments within the two buried, smoke-treated treatments. Control and smoke-treated treatments have been analysed (ANOVA) separately due to the large difference in variances; different letters represent a significant difference (Tukey HSD).
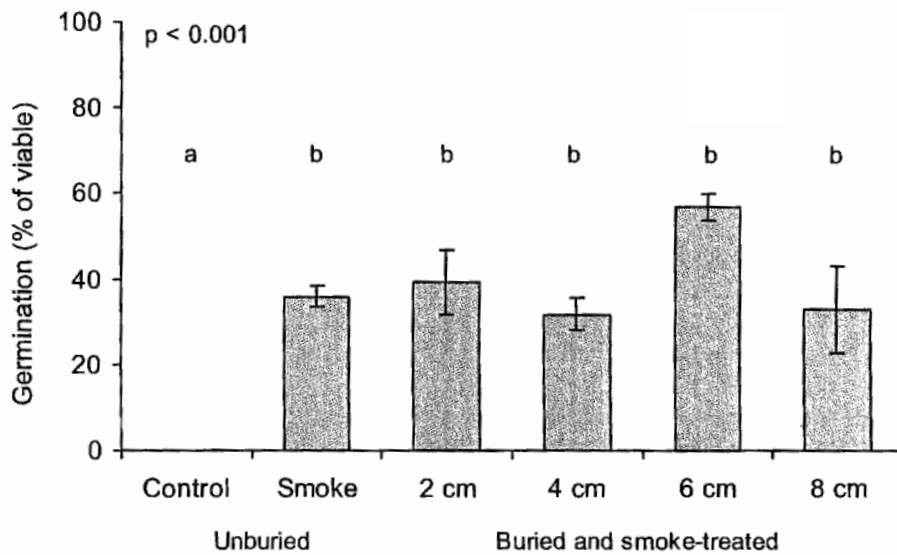
Figure 6.2 Germination response of *Grevillea speciosa* to smoke treatment. Treatment applied to: loose seeds (control, smoke); seeds buried at various depths in sand (2, 4, 6, 8 cm). Error bars are standard error. ANOVA results: *P* value given; different letters represent a significant difference (Tukey HSD).
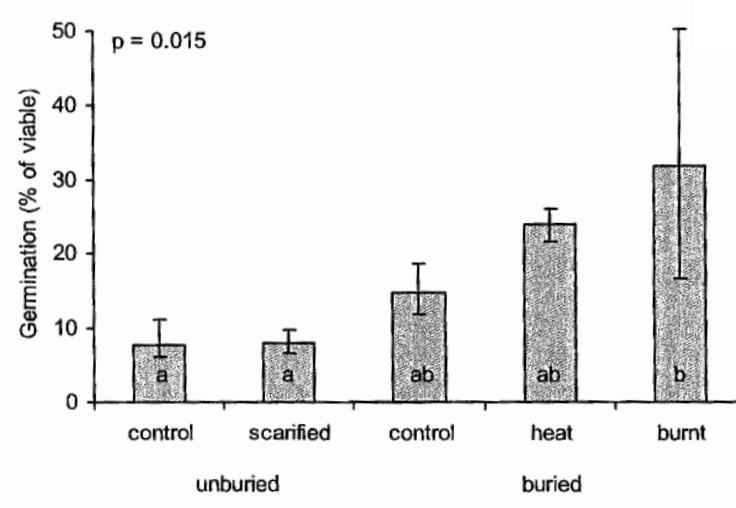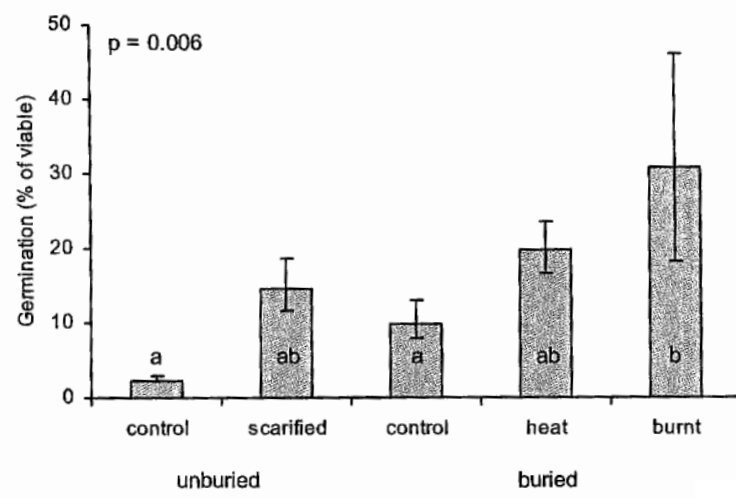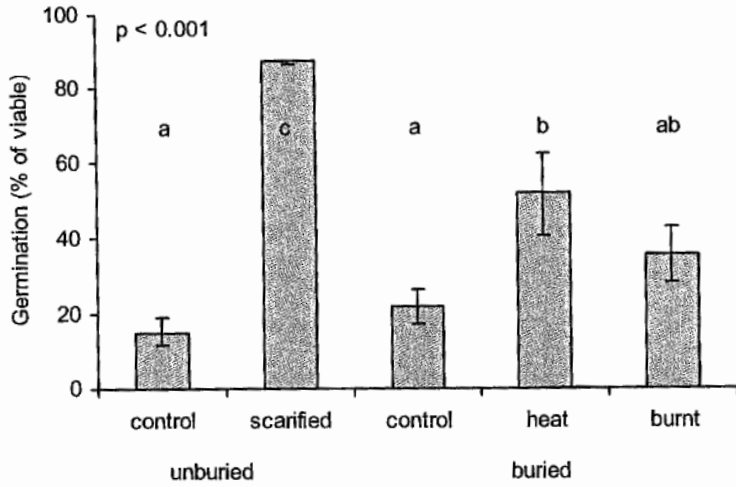
Figure 6.3 Germination responses of (a) *Acacia suaveolens,* (b) *Grevillea sericea,* and (c) *Grevillea speciosa* to fire and fire-cue treatments. Data are back-transformed from arcsine data. Error bars are standard error. ANOVA results: *P* value given; different letters represent a significant difference (Tukey HSD).

Discussion

Smoke Effect on Buried Seeds

Both *Grevillea sericea* and *Grevillea speciosa* showed improved germination with smoke treatment. While control germination was much lower than in previous trials (Chapter 5), the 'smoke effect' (germination level achieved with smoke minus that of the control) was similar: *G. sericea* smoke effect 48.0% and 42.3%; *G. speciosa* 36.1% and 41.4% for these and previous experiments respectively (figures are for smoke application to loose seeds). This shows the repeatability of this effect for these species.

Since its discovery as a germination cue, most experiments on smoke have been concerned with either finding a species' optimal germination cue or looking for the active ingredients of the smoke cue. Very little has been done to investigate how the smoke cue is received by buried seeds. In these experiments, the smoke effect was equivalent in buried seeds compared to directly smoke-treated seeds (Fig. 6.1), showing that burial does not hamper the effectiveness of the smoke cue. Thus smoke can be transported readily through substrate and/or transferred from the substrate onto seeds.

Keeley & Fotheringham (1998a) found that smoke can be transferred to loose seeds by both aqueous leachates (i.e. seeds soaked in smoke-treated water) and gases (i.e. indirect seed exposure to smoke vapours). The action of both methods is implied here for buried seeds, as the smoke cue was still received by seeds buried in dry sand, though water facilitated more effective transport of smoke through the substrate (Fig. 6.1). Both forms of smoke transport occurred within the first week following treatment. Finer time scales than those used here would need to be tested to ascertain if the smoke transfer occurs more or less immediately, or whether gaseous or aqueous transport is quicker.

Under field conditions, sufficient rainfall will need to occur before smoke leachates are transported into the soil. While smoke vapours may be received earlier, seeds are likely to remain quiescent until rainfall allows for adequate moisture to initiate germination, at which stage leachates will also be received. K. Dixon has apparently reported that smoke leachates are distributed through the soil profile within the first 26 mm of rain to fall following a fire (K. Dixon unpublished data, cited in Roche *et al.* 1997b).

Smoke penetrated readily and evenly to 8 cm depth through the substrate tested here (Fig. 6.2). Whether the smoke cue can move as easily through a more tightly packed or finer grained substrate remains untested. However, the results indicate the potential for smoke to affect seeds buried more deeply than soil heating from low-intensity fire does, while the depth-related effects of a heat cue (Auld & Tozer 1995) are not suggested. Whether seeds are actually present and can emerge from these depths will depend on seedbank distribution and seed size. The majority of seeds are found within the top 5 cm of the soil profile, with lesser quantities found down to depths of 10 cm or more (Graham & Hutchings 1988, de Villiers *et al.* 1994, Odion 2000, Grant & MacGregor 2001). The depth from which seedlings can successfully emerge is inversely related to seed size (Bond *et al.* 1999), and the rate of emergence declines rapidly with depth beyond a favourable point (Auld 1986b, Blackshaw 1992). Small seeds may emerge from only the top 2 cm of the soil profile (Bond *et al.* 1999), while larger seeds may emerge easily from depths of up to 8 cm and less successfully from 10 cm (Auld 1986b).

If there is indeed no depth-related variation in germination response to a smoke cue, there would be limited potential for a residual seedbank to remain post-fire in species showing a strong smoke response. Comparative seedbank data for *Acacia suaveolens*, *Grevillea speciosa* and *Grevillea buxifolia* (T. Auld, pers. comm.) suggests this to be the case, with *A. suaveolens* (a heat-responder; Chapters 4 & 5) showing a depth-related residual seedbank not seen in the two *Grevillea* species (which respond to both smoke and heat; Chapter 5). For species with only a heat cue, the seeds that are not triggered to germinate by the level of heating they receive remain dormant and hence available for germination with the next fire. In the event of particularly frequent fires, where immaturity of the standing population may not have allowed for replenishment of the seedbank, a residual seedbank would be advantageous (Pausas 1999). If all smoke-responding seeds are triggered to germinate by a single fire (regardless of whether seedlings can successfully emerge), this residual seedbank and its buffering effect against short inter-fire intervals is not available. This would make species with a smoke response less resilient to frequent fire than those with only a heat response, with obligate seeder species with the strongest smoke response at the most risk of local extinction.

Fire and Fire-cue Effects on Buried Seeds

Smoke and charate treatments were both ineffective in these experiments. Charred wood has previously proven unsuccessful in associated experiments (Chapters 3, 5 and 7) and other Australian studies (Bell *et al.* 1987, Marsden-Smedley *et al.* 1997, Enright & Kintrup 2001). However, smoke is well documented as a germination cue for these two *Grevillea* species (Figs 6.1 & 6.2; Chapter 5; Morris 2000), so it is assumed that the smoke treatment failed in some way to reach the seeds in the field. The actual smoke application used may have been insufficient in either the level of application, or the quantity of smoke created during combustion Alternatively, the time and/or conditions under which they were left buried following treatment may not have allowed for adequate transfer of the smoke cue to the seeds. From the previous experiment, it would be expected that smoke vapours would have been received within the week the seeds were buried. Perhaps the soil the seeds were buried in here did not allow gaseous movement as readily as did the sand in the laboratory experiments, leaving a greater reliance on aqueous transport. During the time that the seeds were left buried at the field site at least one rain event occurred (personal observation at the site on 7-8-99; Table 6.5). However, this may not have been sufficient rain to create smoke leachates for aqueous transport of the smoke cue. It is suggested than in further experiments of this kind, seeds should be left buried in the field until considerable rainfall has been recorded, or that manual watering is performed.

Acacia suaveolens is a hard-seeded species requiring a cue such as heat to break this physical dormancy (Auld 1986b). The seeds respond best to temperatures between 60 and 80 °C, while temperatures greater than 100 °C lead to significant seed mortality (Chapter 4; Auld 1986b). Neither the applied heat treatment nor the prescribed fire led to seed mortality here, while the heat treatment gave significantly higher germination than the fire (Fig. 6.3a). This shows that the soil heating achieved by the prescribed fire was lower than that of the artificially applied treatments (57-94 °C at 1 cm depth). This low level of soil heating, and hence germination of seeds requiring a heat stimulus, is often seen in low-intensity prescribed fires, where temperatures above 60 °C rarely occur beyond a few centimetres depth (Auld 1986b, Bradstock & Auld 1995).

For the *Grevillea* species (Figs 6.3b & c) while heat treatment gave some improvement in germination, this was inferior to the effect of the prescribed fire. This implies that an effect other than heat was produced by the fire. This can perhaps be interpreted as the smoke effect and/or the interactive effect of smoke and heat produced by the fire (see Chapter 5 re multiple cues for *Grevillea* spp.).

Comparison of these experiments with the more controlled laboratory trials of Chapter 5 (Table 6.6) shows that treatment effects were considerably lower when performed under field conditions. It is also seen that both the heat and inferred smoke effect of the prescribed fire were lower than the potential indicated by laboratory trials. Laboratory trials indicated that with optimal treatment germination of all of these species was greater than 70% (71-96%), while the prescribed fire only gave c. 30% (26-34%) germination.

As obligate seeders with soil seedbanks (Chapter 2) these species are reliant on post-fire seedling recruitment for population replacement. Such low levels of germination and hence recruitment following low-intensity prescribed burning may lead to population decline of both these and functionally similar species (Auld & O'Connell 1991, Keith 1996). The concern expressed above regarding the lack of residual seedbank for smoke-responding species does not appear to be a problem in this instance, as the germination level even where a smoke effect was implied was low (note that this may have been higher if more aqueous smoke had been allowed to penetrate the soil with greater rainfall at the site). There is potential for the smoke effect to be quite variable within a fire, perhaps allowing for residual seedbank to be left in areas of soil receiving minimal smoke vapours and/or leachates. This would lead to greater spatial variation in the distribution of the residual seedbank compared to heat-only responders which also have a depth-related residual.

If a particular recruitment result is considered desirable from a prescribed fire, it may be possible to manipulate the physical effects of the fire to suit the germination-cue requirements of the target species. Soil heating produced by a fire varies with fuel quantity, fuel distribution, fuel moisture, prevailing weather conditions (Raison 1979), fire intensity, fire duration, soil texture, and soil moisture (Auld & O'Connell 1991). The quantity of smoke produced will depend on the fuel load, fuel moisture and burning rate (Vines *et al.* 1971), with slow-moving, smouldering fires producing the most smoke (Jager *et al.* 1996a, Keeley & Fotheringham 1998a). The degree to which these factors can be manipulated is constrained by various logistic (resource and staff availability; Bradstock *et al.* 1998b), safety (fire controllability; Bradstock & Auld 1995) and legal (smoke pollution management; NSW National Parks and Wildlife Service 2001) issues.

Table 6.5 Regional rainfall records for the period that seeds were buried in the field at Heathcote. Bureau of Meteorology records for Sydney (Observatory Hill station) and Wollongong (Wollongong University station). These are the two nearest automatic weather stations for which data was available for the time period; the field site is located between these two stations.

Date	Rainfall recorded (mm)	
	Sydney	Wollongong
6-8-99	0.0	0.0
7-8-99	32.0	8.2
8-8-99	0.0	0.1
9-8-99	0.0	0.6
10-8-99	0.0	0.0
11-8-99	0.0	0.0
12-8-99	0.0	0.0

Table 6.6 Comparison of heat and smoke effect between buried (fire and fire-cue experiment, this Chapter) and loose (Chapter 5) seeds. Germination as percentage of viable seed (raw data). Fire effect = burnt – buried control; heat effect = heated treatments – buried control (heat treatment – control for loose seeds); residual (inferred smoke effect) = fire effect – heat effect; smoke effect = smoke treatment – control (loose seeds).

Species	Buried seeds (this experiment)			Loose seeds (Chapter 5)	
	Fire effect	Heat effect	Residual	Heat effect	Smoke effect
Acacia suaveolens	19.2	45.9	-26.7	62.7	5.2
Grevillea sericea	23.3	7.4	15.9	22.4	42.3
Grevillea speciosa	21.8	8.2	13.6	22.7	41.4

CHAPTER 7: GERMINATION CUE EFFECTS ON A NATURAL SOIL SEEDBANK

Aim

The previous chapters have reported germination responses from trials performed on freshly collected seeds. This chapter looks at the same fire-related cues (smoke, charred wood and heat) applied to natural seedbanks, under both glasshouse and field conditions, and includes a comparison of the effects on seed germination of a fire versus individually applied fire-related treatments. Studies on natural seedbanks suffer from the uneven distribution of seeds, thus without knowing what seeds were originally in each sample it can be hard to draw definitive conclusions about the effects of treatments on individual taxa. However, since seeds may behave differently following soil storage (Roche *et al.* 1997a, Tieu & Egerton-Warburton 2000), seedbank studies may give a more realistic picture of germination effects in nature (Odion 2000).

Methods

Soil used in this chapter was collected from two sites within the Bobbin Head section of Ku-ring-gai Chase National Park (Sites 15 and 16 as shown on Maps 2.2 & 7.1). The vegetation of both sites is classed as low woodland/low open-woodland (map unit 15 of Thomas & Benson 1985) with *Eucalyptus gummifera* and *E. haemastoma* as dominant canopy species and a diverse sclerophyllous shrub understorey. This falls under the Sydney Sandstone Ridgetop Woodland (map unit 10ar) classification of Benson & Howell (1994). Average soil depth for this formation is 5-10 cm (Pidgeon 1938). The recent (from 1960; some records from the 1940s) fire history of Ku-ring-gai Chase National Park is recorded by the NSW National Parks and Wildlife Service (Conroy 1996). Prior to sampling in 1998, Site 15 was last burnt by wildfires in 1990 and 1974; Site 16 was last burnt by wildfires in 1983 and 1977.

Pilot Glasshouse Trial

Soil was collected from Ku-ring-gai Chase National Park (Site 15; Map 7.1) in July 1998. Soil was collected using a manual soil corer of 10 cm diameter. Leaf litter was gently scraped away from the soil surface before the soil corer was pushed into the soil to a depth of 5 cm, and then carefully levered back out containing a column of soil. Thirteen bags of soil were collected, each bag containing ten soil cores. Each bag of soil was weighed (moist weight) before and after coarse sieving to remove stones, twigs and leaves. Mean weight of soil per bag after sieving was 4.72 kg. Soil from all bags was mixed to randomise seed occurrence, and 3 kg sub-samples were taken from this bulked soil for each treatment replicate. Each soil sub-sample represented an area of 0.05 m^2.

Treatments were applied to individual 3 kg soil sub-samples. Four treatments were applied: control, heat (oven pre-heated to 80 °C, heated for 20 minutes), smoke (litter material from the unburnt site burnt in a bee-keeper's burner; aerosol smoke applied for 20 minutes), and heat-smoke combined (heat applied before smoke). Five replicates per treatment were treated separately.

Each treated soil replicate was spread evenly in a seedling tray (320×270 mm) in a temperature-controlled glasshouse. Trays were arranged randomly in the glasshouse and briefly watered following treatment.

Watering was then performed automatically every morning for 3 minutes. Two trays of river sand were placed amongst the soil trays to measure seed rain contamination.

Seedling emergence was monitored regularly (at least once per week) for a four-month period. Trays were randomly rearranged after two months.

Myall Track Prescribed Burn

A prescribed fire was performed on the 29th of October 1998 by the NSW National Parks and Wildlife Service within Ku-ring-gai Chase National Park, in an area of c. 25 ha bounded by Ku-ring-gai Chase Road, the works depot road and the Myall Track (Site 16; Map 7.1). A control line was put in place around an area to be left unburnt within the boundary of this fire.

One week after the fire burnt and unburnt sub-sites were located in adjacent areas on either side of the control line, separated by approximately 15 m. A plant species list was collated from within the unburnt sub-site.

Plots for field monitoring of emergence from the seedbank were set up and treated in the second and third week after the fire. Monitoring of these plots commenced on the 30th of November, one month after the fire. At this time, soil was also collected for a complementary glasshouse analysis of the seedbank. Species that had already emerged within the burnt sub-site by this time were noted and later identified, as these species were likely to have already germinated within the soil collected and hence may have affected the results of the glasshouse study. These species were *Acacia myrtifolia*, *Bossiaea heterophylla*, *Eucalyptus* spp., and *Gompholobium glabratum*.

Once both seedbank studies (glasshouse and field plots) were completed, the floristic similarity between the standing vegetation and the seedbank was calculated using the Jaccard similarity coefficient. The species list used for the seedbank was compiled by combining results from both the glasshouse and field plots in order to account for all species encountered from the seedbank regardless of species' preferences for the different methods. Unknown and aggregated species were excluded from the species list. Similarity was not calculated between the glasshouse and field seedbank studies as different treatments were applied, the amount of soil used represented a different area, and environmental conditions differed between the field and glasshouse.

Note that this experiment was performed within a single area of a single fire. Thus for both the glasshouse and field experiments, there is no true replication of the 'burnt' treatment (in comparison to the treatments applied to the unburnt soil, which were all performed independently as per Morrison and Morris (2000)). This is a common problem of oppurtunistic post-fire studies. However, the small-scale heterogeneity evident within any one fire event (Atkins & Hobbs 1995, Odion & Davis 2000, Catchpole 2002) provides for some random variation of the 'experimental treatment'.
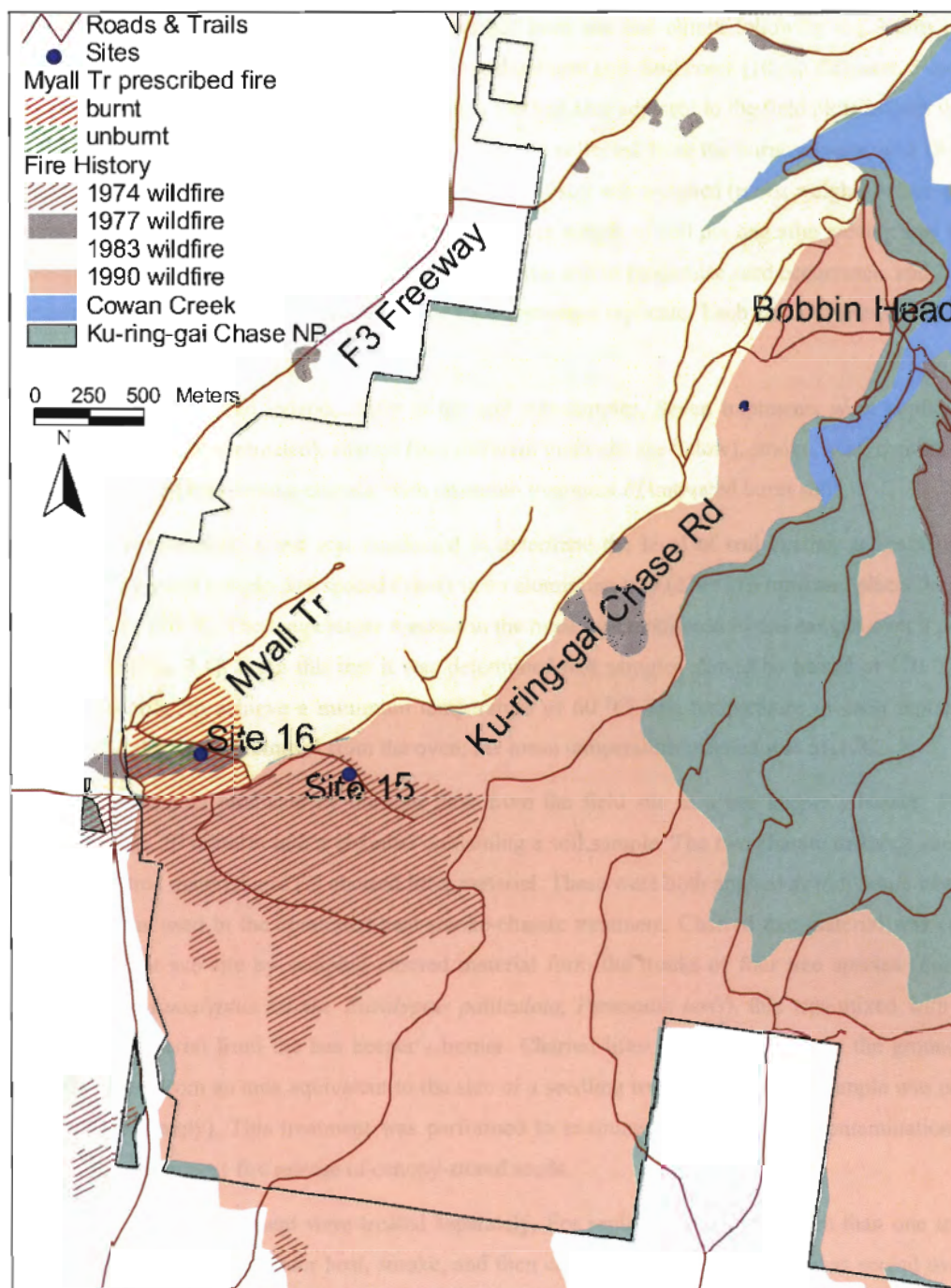
Map 7.1 Bobbin Head section of Ku-ring-gai Chase National Park showing sites for soil collection and field work: Site 15 = pilot glasshouse study; Site 16 = Myall Track prescribed burn site, glasshouse study and field plots. Fire history relevant to Sites 15 and 16 is shown (from NSW National Parks & Wildlife Service records); the prescribed fire is divided into the burnt and unburnt sections.

(A) Glasshouse Trial

Soil was collected from the Myall Track prescribed burn site one month following the fire to compare seedling emergence from burnt, unburnt and treated unburnt soil. Soil cores (10 cm diameter, 5 cm depth; collected as described above) were taken within a 10×5 m area adjacent to the field plots in both the burnt and unburnt sub-sites. Three bags of 10 soil cores were collected from the burnt sub-site, and 18 bags of 10 cores from the adjacent unburnt sub-site. Each bag of soil was weighed (moist weight) before and after coarse sieving to remove stones, twigs and leaves. Mean weight of soil per bag after sieving was 4.81 kg. Soil from all bags was aggregated into burnt and unburnt soil to randomise seed occurrence, and 2 kg sub-samples were taken from this bulked soil for each treatment replicate. Each soil sub-sample represented an area of 0.033 m^2.

Treatments were applied independently to the soil sub-samples. Seven treatments were applied to the unburnt soil: control (untreated), charate (two different methods, see below), smoke, heat, combined heat-smoke, combined heat-smoke-charate; with an eighth treatment of untreated burnt soil.

Prior to heat treatment, a test was conducted to determine the level of soil heating achieved within a sample. A 2 kg soil sample was spread evenly in an aluminium tray (275×215 mm) and placed in an oven pre-heated to 120 °C. The temperature reached in the oven was monitored in this sample over a period of 60 minutes (Fig. 7.1). From this test it was determined that samples should be heated at 120 °C for 40 minutes in order to achieve a minimum temperature of 60 °C. The temperature of each replicate was measured at the time of removal from the oven; the mean temperature reached was 61.1 °C.

Aerosol smoke was produced by burning litter from the field site in a bee keeper's burner. This was channelled for 20 minutes into a chamber containing a soil sample. The two charate methods used were: (1) charred tree material and (2) charred litter material. These were both applied as individual treatments; method 1 was used in the combined heat-smoke-charate treatment. Charred tree material was collected from the burnt sub-site by scraping charred material form the trunks of four tree species (*Eucalyptus haemastoma, Eucalyptus eximia, Eucalyptus paniculata, Persoonia levis*), this was mixed with freshly burnt litter material from the bee keeper's burner. Charred litter was collected from the ground in the burnt sub-site, from an area equivalent to the size of a seedling tray (each replicate sample was collected and kept separately). This treatment was performed to examine the influence of contamination of this charate source by post-fire release of canopy-stored seeds.

Five replicates per treatment were treated separately. For replicates receiving more than one treatment these were applied in the order heat, smoke, and then charate. Each soil replicate was spread over 1 cm depth of sand in a seedling tray (320×270 mm). Trays were arranged randomly in the glasshouse after treatment and briefly watered. Watering was then performed automatically every morning and afternoon for 1 minute. Two trays of river sand were placed amongst the soil trays to measure seed rain contamination.

Seedling emergence was monitored regularly (at least once per week) for a five-month period. Species not yet identified by the end of the trial were re-potted and allowed to continue growing.

Several seedlings were found already germinated in the burnt soil before it was placed in the trays in the glasshouse and were discarded. These were *Eucalyptus* spp., Fabaceae spp. and a monocotyledon species.

(B) Field Plots

Plots to monitor seedling emergence in the field were set up at the Myall Track site within three weeks of the fire, also to compare burnt, unburnt and treated unburnt soil. Fifty 0.25 m^2 square plots (10 rows each of five plots over an area of 16×13 m) were set up in the unburnt sub-site to which treatments were applied. Five treatments were applied in a randomised block design, with one plot per row of each treatment. Treatments applied were: control 1 (uncleared), control 2 (cleared), charate, smoke, combined charate-smoke.

All plots (except control 1) were cleared of vegetation and litter prior to treatment application. Charate treatment was equivalent to the charred litter treatment used in the glasshouse trial; charred litter was collected from the ground in the burnt sub-site from a 0.25 m^2 area and spread evenly over the plot. A 'smoke tent' was constructed from lined thick calico material, to cover an area of 0.25 m^2. The 'smoke tent' was secured with tent pegs over the plot, and smoke channelled into this from the beekeeper's burner. Litter material from the site was burnt in the burner, and smoke treatment was applied for a period of 20 minutes. Plots receiving the combined treatment had smoke applied before the charate.

Ten plots were also established in the burnt sub-site, placed randomly over an equivalent sized area (16×13 m). No treatments were applied to these plots.

Seedling emergence was monitored regularly (approximately once per month) for a 13-month period. Rainfall for Sydney (Observatory Hill station; Bureau of Meteorology climate records) during this period is shown in Figure 7.2. Observatory Hill was the closest automatic weather station recording rainfall over this time period; the study site is approximately 20 km NNE of Observatory Hill.
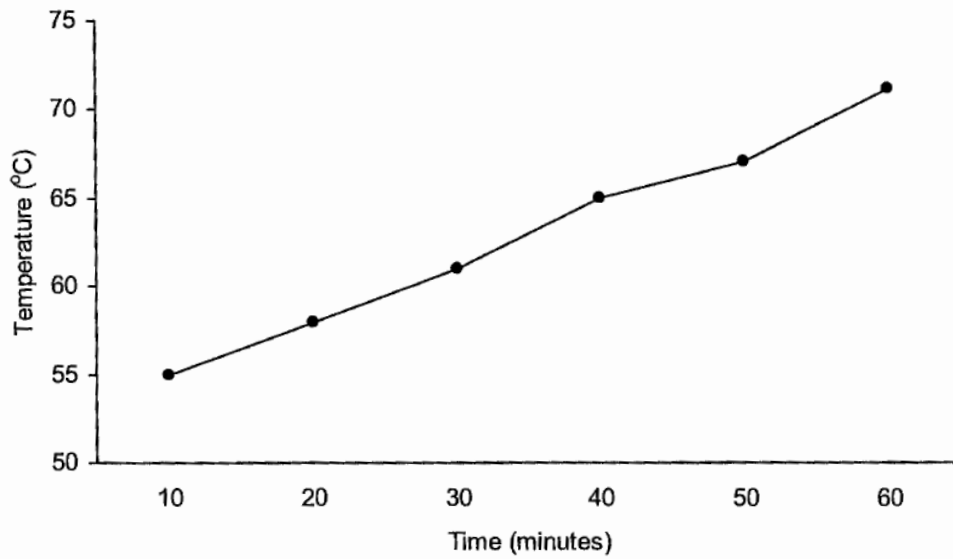
Figure 7.1 Temperature reached over time within a 2 kg soil sample in an oven pre-heated to 120 °C.
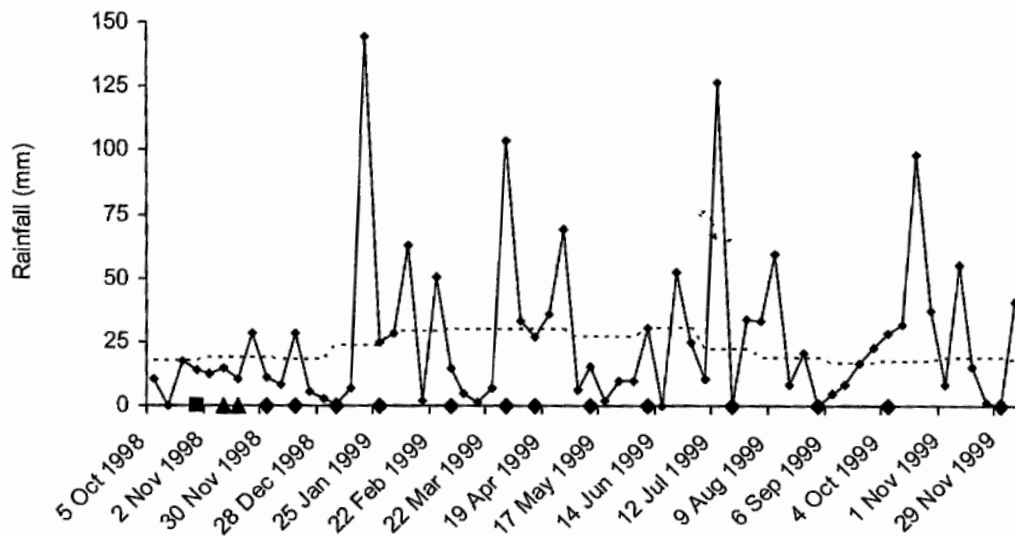
Figure 7.2 Rainfall for Sydney station for the duration of field plot monitoring: solid line = actual weekly rainfall; dotted line = average weekly rainfall (historical). Symbols on x axis represent field activity: square = date of prescribed fire; triangle = date of plot set up and treatment; diamond = monitoring dates.

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Statistical Analysis

Species richness and seedling density were analysed for treatment effects by one-way analysis of variance (ANOVA) and post hoc Tukey honestly significant difference (HSD) multiple comparison. The incomplete experimental design meant that a fully factorial ANOVA could not be used. Richness and density were analysed both as total seedling emergence and with seedlings divided into three categories: seedlings from the soil seedbank, seedlings of species with tubers (these were separated from the soil seedbank species as it appeared that once an individual emerged vegetative propagation may have occurred, perhaps falsely increasing the number of 'germinants' counted), and seedlings from canopy-stored seedbanks (these occurred predominantly in the burnt soil, where canopy stored seeds were shed post-fire, and in the charred litter treatments which received these seeds via contamination of the charate source).

Individual species with a reasonable quantity of seedlings (a mean of at least one seedling per tray or plot) were also analysed for treatment effects by one-way ANOVA and post hoc Tukey HSD tests.

Prior to ANOVA, data were checked for homogeneity of variance using Cochran's test. Most data required log transformation ($\ln(x+1)$) to improve homogeneity. For two species (*Micrantheum ericoides* and *Eucalyptus* spp.) in the field plots, log transformation did not improve homogeneity sufficiently to satisfy Cochran's test at the 0.05 level, but were satisfactory at the 0.01 level; hence for these ANOVAs the significance level was reduced to 0.01. Where data has been log transformed, back-transformed data are presented in Figures.

Results

Pilot Glasshouse Trial

A total of 151 seedlings (115 dicots, 36 monocots) emerged across all trays. Species richness was low, with eight species distinguishable, and several more unidentified (Table 7.1).

Seedling density varied with treatment for total seedling emergence ($P = 0.004$) and for dicotyledon seedlings ($P = 0.001$), but not for monocotyledons ($P = 0.617$). The pattern of seedling density was the same for dicotyledons as for total emergence (Fig. 7.3), with only heat and smoke combined resulting in a significantly greater seedling density than the control. The effect of smoke alone was intermediate between control and the heat-smoke combination (Tukey HSD).

Individual species with more than 20 seedlings (represents a mean of at least one seedling per tray) were analysed separately for treatment effects. For *Actinotus minor* the overall ANOVA result showed no significant pattern ($P = 0.056$), however the Tukey test picked up a difference between control and combined heat-smoke (Fig. 7.4a). *Epacris pulchella* showed significantly greater emergence ($P = 0.001$) in treatments receiving smoke compared to control, and a trend for a combined heat-smoke response (Fig. 7.4b).

Table 7.1 Number of seedlings emerging across all treatments, pilot glasshouse trial.

Species	Number of seedlings
Actinotus minor	26
Drosera sp.	7
Epacris pulchella	43
Eucalyptus spp.	1
Grevillea sericea	1
Pultenaea sp.	11
unidentified dicots	26
Lomandra sp.	7
unidentified grass	23
unidentified monocots	6
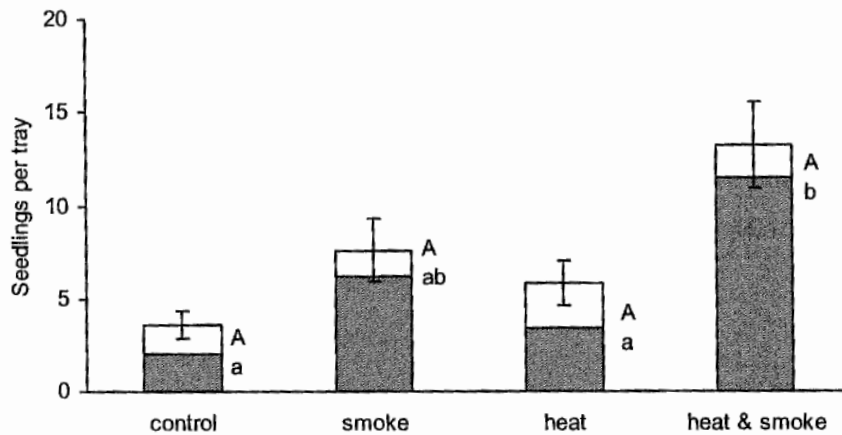
Figure 7.3 Total seedling emergence in the pilot glasshouse trial; raw data: grey bars = dicotyledons; white bars = monocotyledons. Error bars are standard error of total emergence. ANOVA results shown: different symbols represent a significant difference (Tukey HSD) for dicotyledon (ANOVA $P = 0.001$; lower case letters) and monocotyledon (ANOVA $P = 0.617$; capital letters) species respectively.
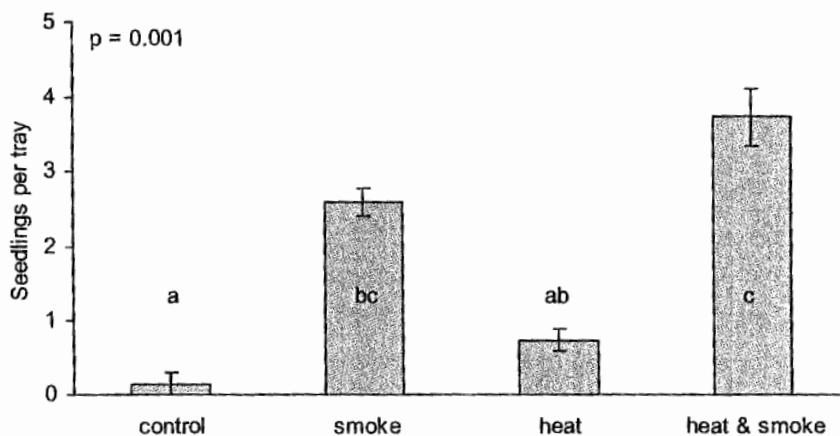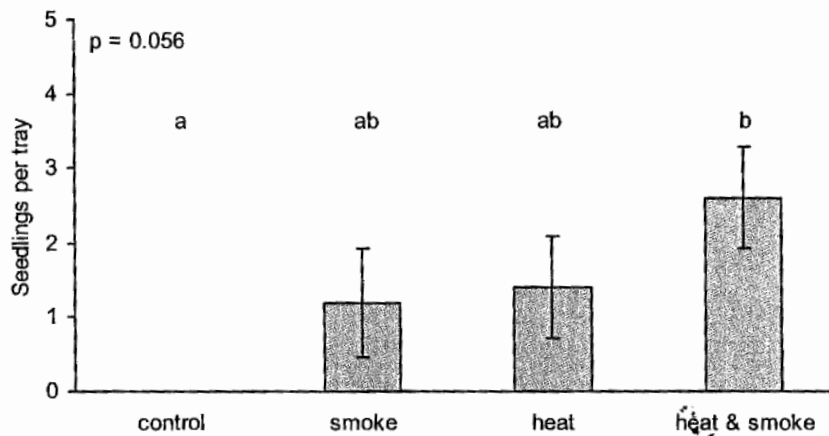
Figure 7.4 Emergence of (a) *Actinotus minor* and (b) *Epacris pulchella* in the pilot glasshouse trial. Error bars are standard error. ANOVA results shown: P value given; different letters represent a significant difference (Tukey HSD).

Myall Track Prescribed Burn

A list of species recorded in the standing vegetation at the site (unburnt sub-site) and the number of seedlings emerging in the two seedbank studies (glasshouse and field plots) is given in Table 7.2.

The Jaccard similarity coefficient between species seen in the standing vegetation and the seedbank (glasshouse and field plots combined) was 55%. Of the 53 species considered for this analysis (unknown and aggregated species were excluded), 13 species were seen only in the standing vegetation, 11 species only in the seedbank and 29 species were common to both.

(A) Glasshouse Trial

A total of 878 seedlings emerged across all 40 trays, consisting of 764 seedlings from the soil seedbank, 64 seedlings of species with tubers (Orchidaceae spp. and *Drosera peltata;* these were separated from the soil seedbank species as it appeared that once an individual emerged vegetative propagation may have occurred, perhaps falsely increasing the number of 'germinants' counted), and 50 seedlings from canopy stored seedbanks (these occurred predominantly in the burnt soil, where canopy stored seeds were shed post-fire, and in the charred litter-treated trays which received these seeds via contamination of the charate source). Seedlings of 39 species were distinguished, though a few of these remained unidentified (due to death of the seedlings before identification could be made). One species identified as a weed contaminate from elsewhere in the glasshouse was removed when encountered and not included in seedling tallies.

Species richness (Fig. 7.5a) of total seedling emergence varied between treatments ($P < 0.001$), reflecting the influence of both soil seedbank ($P < 0.001$) and canopy seedbank ($P < 0.001$) species. Species with tubers showed no difference between treatments ($P = 0.426$). Species richness amongst soil seedbank species was greatest in heat-treated trays, with the charate treatment having no effect, and the smoke-only treatment being intermediate between the control and heat-treated trays. Canopy seedbank species were only prominent in the charred litter and burnt treatments. Overall species richness was unaffected by the charred wood treatment and was highest in the burnt soil.

Few species were unique to only one treatment: two unique species in charred litter, one in heat, one in heat-smoke, three in burnt (Table 7.2). These species all occurred in very low quantity (one or two seedlings total) except for *Leptospermum trinervium* (10 seedlings emerged across all burnt treatment trays).

Seedling density (Fig. 7.5b) of total seedling emergence varied between treatments ($P < 0.001$), reflecting the influence of both soil seedbank ($P < 0.001$) and canopy seedbank ($P < 0.001$) species. Species with tubers showed no difference between treatments ($P = 0.347$). Seedling density amongst soil seedbank species was unaffected by charate or smoke treatment alone, but was significantly greater with all heat treatments and burnt soil. Canopy seedbank species were only prominent in the charred litter and burnt treatments. Overall seedling density was unaffected by charred wood or smoke treatment and was highest in heat-treated soil.

Species with nearly 40 or more seedlings (representing a mean of one seedling per tray) were analysed individually for treatment effects.

Drosera peltata showed no significant difference ($P = 0.403$) between treatments. *Actinotus minor* (Fig. 7.6a; $P < 0.001$) emergence was only significantly enhanced in heat-treated soil. *Epacris pulchella* (Fig. 7.6b; $P < 0.001$) and *Entolasia stricta* (Fig. 7.6c; $P < 0.001$) were also unaffected by the charate and smoke treatments, but had significantly greater emergence from both heat-treated and burnt soil. *Patersonia sericea* (Fig. 7.6d; $P < 0.001$) had greatest emergence in the heat-alone treatment. The other heat-treated trays were intermediate between this and the control. Smoke and charate only treatments, and burnt soil, had no significant effect.

Table 7.2 Species list for Myall Track prescribed burn site. Seedbank type (SB) is given for each species: C = canopy stored, S = soil stored, T = transient seedbank. Species recorded in the standing vegetation (veg) at the site (unburnt sub-site) are marked with an asterisk. The total number of seedlings emerging per treatment is given for (a) glasshouse trays: C = control, Ch = charred wood, Ch2 = charred litter, S = smoke, H = heat, HS = heat & smoke, HSC = heat, smoke & charate, B = burnt; (b) field plots: C = control, Cl = cleared, Ch = charate, S = smoke, SC = smoke & charate, B = burnt. NB Some germination occurred within the burnt soil samples prior to placement in the glasshouse, hence the density of these species (marked with †) is underestimated in this treatment.

Species	SB	Veg	Glasshouse								Field					
			C	Ch	Ch2	S	H	HS	HSC	B	C	Cl	Ch	S	SC	B
Lindsaeaceae																
Lindsaea linearis	S	*														
Iridaceae																
Patersonia sericea var. *sericea*	S	*	2	3			2	16	8	8						
Lomandraceae																
Lomandra sp.	S	*														
Lomandra glauca	S	*	1						1							
Lomandra obliqua	S	*														
Orchidaceae																
Corybas sp.	S				1						2	2	8		10	
Orchidaceae sp.	S												1	1	1	5
Poaceae																
Entolasia stricta	S	*	1	1			2	27	21	26†	12					
Restionaceae																
Hypolaena fastigiata	S	*														
Leptocarpus tenax	S				1				1							
Xanthorrhoeaceae																
Xanthorrhoea media	T	*														
Xyridaceae																
Xyris sp.	S	*														
unknown monocots	S		4	1			1		3							
Apiaceae																
Actinotus minor	S	*		3	3	8	30	28	46	4	1	9	108	69	101	47
Platysace linearis	S	*												1		
Casuarinacaeae																
Allocasuarina sp.	C														1	
Dilleniaceae																
Hibbertia sp.	S	*														
Droseraceae																
Drosera peltata	S	*	13	7	19	9	5	3	2	5	1	15	34	35	25	
Epacridaceae																
Epacris pulchella	S	*		2	1	4	86	94	104	48		13	62	104	121	39
Monotoca scoparia	S	*														
Euphorbiaceae																
Micrantheum ericoides	S	*			1		3	1	1	17	3	4	1	2	14	35
Phyllanthus hirtellus	S						1	1								5

Species	SB	Veg	Glasshouse								Field					
			C	Ch	Ch2	S	H	HS	HSC	B	C	Cl	Ch	S	SC	B
Fabaceae																
Acacia myrtifolia	S				1					1†		10	3		1	14
Bossiaea heterophylla	S	*						1				3	7	9	13	14
Gompholobium glabratum	S	*								1			3			28
Platylobium sp.	S		1				1	2								
Pultenaea sp.	S	*			1			1		†		1		2		1
Goodeniaceae																
Dampiera stricta	S	*											3		1	3
Goodenia sp.	S	*														
Haloragaceae																
Gonocarpus sp.										2	1					10
Lamiaceae																
Hemigenia purpurea	S	*				1	4	2	1				5	4	6	8
Lauraceae																
Cassytha glabella	S	*			5		1									
Cassytha pubescens	S						1									
Myrtaceae																
Angophora hispida	C	*														
Eucalyptus eximia	C	*														
Eucalyptus haemastoma	C	*														
Eucalyptus spp.	C			1	24				1	5†	1	4	12	5	15	26
Darwinia biflora	S	*		1		1		6	4	1				2		2
Kunzea capitata	S	*			1		1			1						
Leptospermum trinervium	C	*								10						
Proteaceae																
Banksia ericifolia	C	*														
Banksia serrata	C	*														
Banksia spinulosa	C	*			4					1						
Banksia ericifolia/spinulosa	C										2	1				9
Conospermum longifolia	S	*													1	
Grevillea buxifolia	S	*														
Grevillea sericea	S	*					2		1							
Grevillea buxifolia/sericea	S												2	7	2	5
Hakea dactyloides	C	*														
Hakea teretifolia	C	*													2	2
Lambertia formosa	C	*														
Persoonia lanceolata	S	*							1							
Persoonia levis	S	*														
Persoonia pinifolia	S	*														2
Petrophile pulchella	C	*			3					1	1					11
Proteaceae spp.	S												1		1	1
Rutaceae																
Boronia pinnata	S	*												1	2	1
Solanaceae																
Solanum sp.	S				1										2	5
Thymelaeaceae																
Pimelea linifolia	S										1					
Tremandraceae																
Tetratheca sp.	S	*							1					2	1	1
unknown dicots	S		5	3	5	6	12	18	21	17	1	10	12	17	32	34

Figure 7.5a Species richness per treatment in the glasshouse trial; raw data: dark grey bars = soil seedbank species; light grey bars = tuber species; white bars = canopy seedbank species. Error bars are standard error of total seedling emergence. ANOVA results shown: different symbols represent a significant difference (Tukey HSD) for soil seedbank (ANOVA $P < 0.001$; lower case letters), tuber (ANOVA $P = 0.426$; capital letters), and canopy seedbank (ANOVA $P < 0.001$; Greek letters) species respectively.
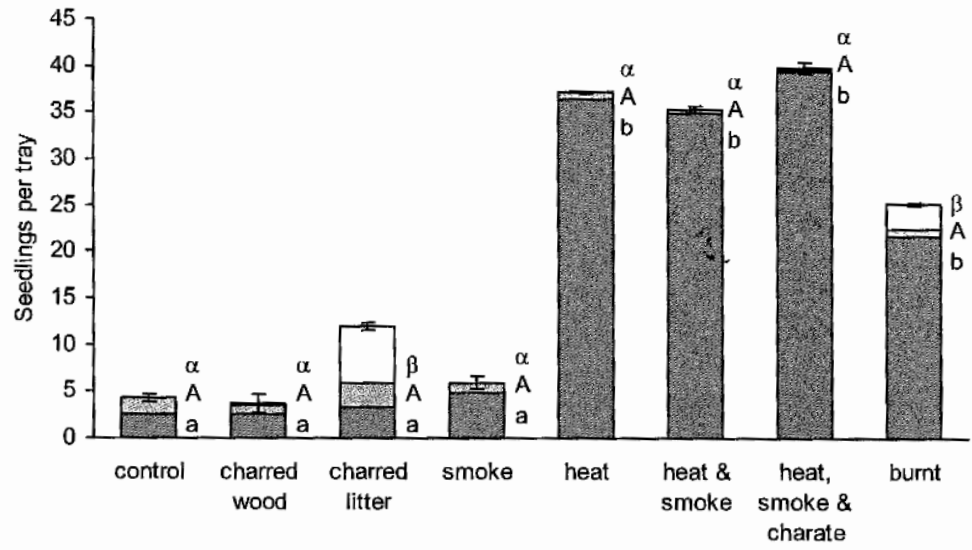
Figure 7.5b Total seedling emergence per treatment in the glasshouse trial; back-transformed data: dark grey bars = soil seedbank species; light grey bars = tuber species; white bars = canopy seedbank species. Error bars are standard deviation of total seedling emergence. ANOVA results shown: different symbols represent a significant difference (Tukey HSD) for soil seedbank (ANOVA $P < 0.001$; lower case letters), tuber (ANOVA $P = 0.347$; capital letters), and canopy seedbank (ANOVA $P < 0.001$; Greek letters) species respectively.
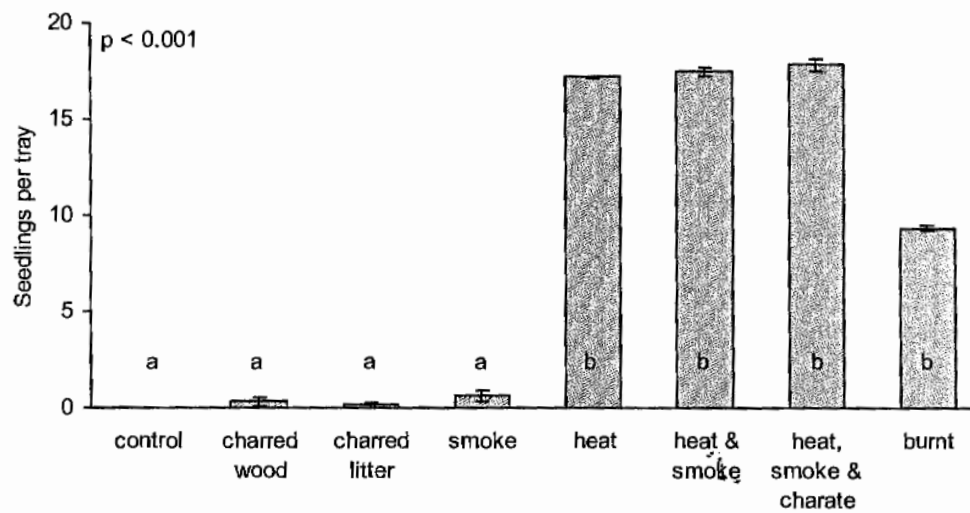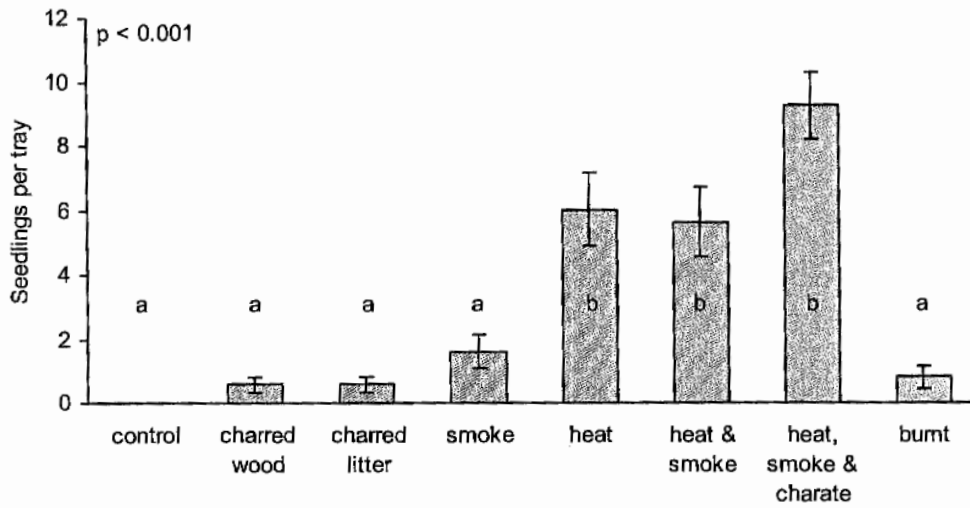
Figure 7.6 Emergence of (a) *Actinotus minor* and (b) *Epacris pulchella* in the glasshouse trial. Error bars are standard error. ANOVA results shown: *P* value given; different letters represent a significant difference (Tukey HSD).

Figure 7.6 continued Emergence of (c) *Entolasia stricta* and (d) *Patersonia sericea* in the glasshouse trial; back-transformed data. Error bars are standard error. ANOVA results shown: *P* value given; different letters represent a significant difference (Tukey HSD).

(B) Field Plots

A total of 1269 seedlings emerged across all 60 plots, consisting of 1037 seedlings from the soil seedbank, 140 seedlings of species with tubers, and 92 seedlings from canopy-stored seedbanks. Seedlings of 40 species were distinguished, though not all of these were identified.

Species richness (Fig. 7.7a) of total seedling emergence ($P < 0.001$) showed the same pattern as that of soil seedbank species ($P < 0.001$), with highest species richness seen in the burnt plots, lower richness seen in the treated plots (charate, smoke, and smoke-charate) and no significant difference between the control and cleared plots. ANOVA results for tuber species were ambiguous, with an overall P value of 0.031, but no differences distinguished by the Tukey HSD test. Canopy seedbank species ($P < 0.001$) had highest species richness in the burnt plots, followed by the charate-treated plots. Other treatments showed no difference compared to the control.

Few species were unique to one treatment: one unique species in control, one in smoke-charate, two in burnt (Table 7.2). These species all occurred in very low quantity (one or two seedlings total).

Seedling density (Fig. 7.7b) of total seedling emergence ($P < 0.001$) showed the same pattern as that of the soil seedbank species ($P < 0.001$), with all treatments except cleared plots having greater seedling emergence than the control, but no difference among the smoke, charate and burnt treatments. ANOVA results for tuber species were ambiguous, with an overall P value of 0.035, but no differences distinguished by the Tukey HSD test. Canopy seedbank species ($P < 0.001$) had highest seedling emergence in the burnt plots, followed by the charate-treated plots. Other treatments showed no difference compared to the control.

Species or families with nearly 60 or more seedlings (representing a mean of one seedling per plot) were analysed individually for treatment effects.

Drosera peltata showed no significant difference ($P = 0.132$) between treatments.

Actinotus minor (Fig. 7.8a; $P < 0.001$) had greatest emergence in the smoke, charate and smoke-charate treated plots. Emergence in the burnt plots was intermediate between these and control and cleared plots. *Epacris pulchella* (Fig. 7.8b; $P = 0.002$) had greatest emergence in the smoke, charate and smoke-charate treated plots. Emergence in burnt and cleared plots was intermediate between these and the control.

Micrantheum ericoides (Fig. 7.8c; $P = 0.001$) had greatest emergence in the burnt plots, other treatments having little effect. *Eucalyptus* spp. (Fig. 7.8d; $P < 0.001$) had greatest emergence in the burnt and charate-treated plots. NB The significance level for these two genera was lowered to 0.01 due to the variance.

The combined Fabaceae species (Fig. 7.8e; $P < 0.001$) had greatest emergence in the burnt plots, and were unaffected by all other treatments.
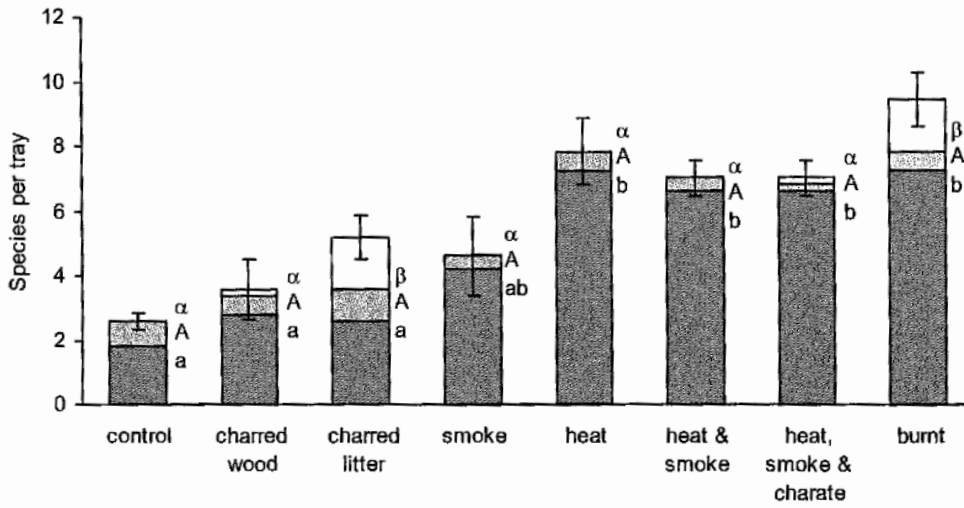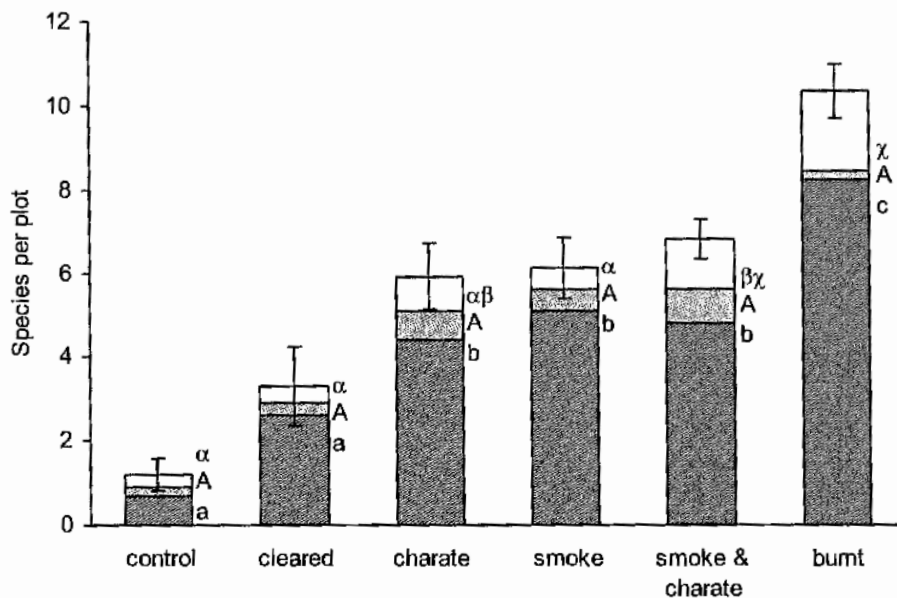
Figure 7.7a Species richness per treatment in the field plots; raw data: dark grey bars = soil seedbank species; light grey bars = tuber species; white bars = canopy seedbank species. Error bars are standard error of total species richness. ANOVA results shown: different symbols represent a significant difference (Tukey HSD) for soil seedbank (ANOVA $P < 0.001$; lower case letters), tuber (ANOVA $P = 0.031$; capital letters), and canopy seedbank (ANOVA $P < 0.001$; Greek symbols) species respectively.
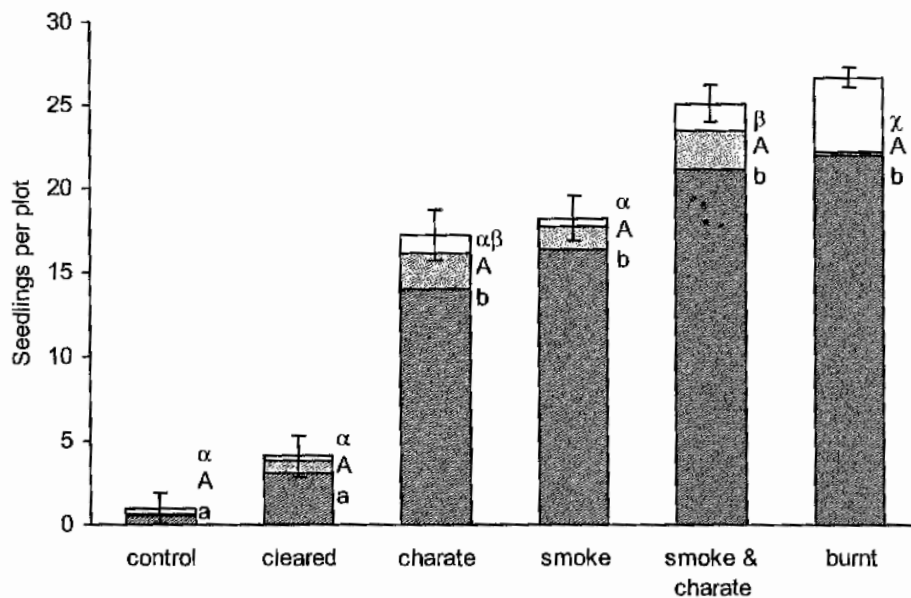

Figure 7.7b Total seedling emergence per treatment in the field plots; back-transformed data: dark grey bars = soil seedbank species; light grey bars = tuber species; white bars = canopy seedbank species. Error bars are standard deviation of total seedling emergence. ANOVA results shown: different symbols represent a significant difference (Tukey HSD) for soil seedbank (ANOVA $P < 0.001$; lower case letters), tuber (ANOVA $P = 0.035$; capital letters), and canopy seedbank (ANOVA $P < 0.001$; Greek letters) species respectively.

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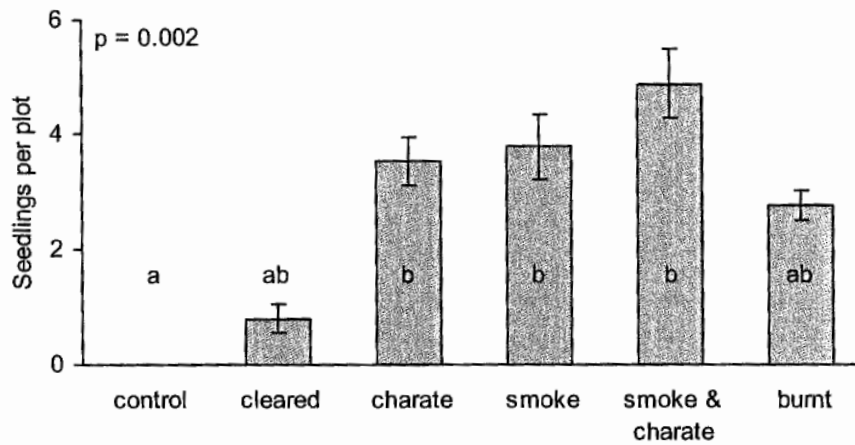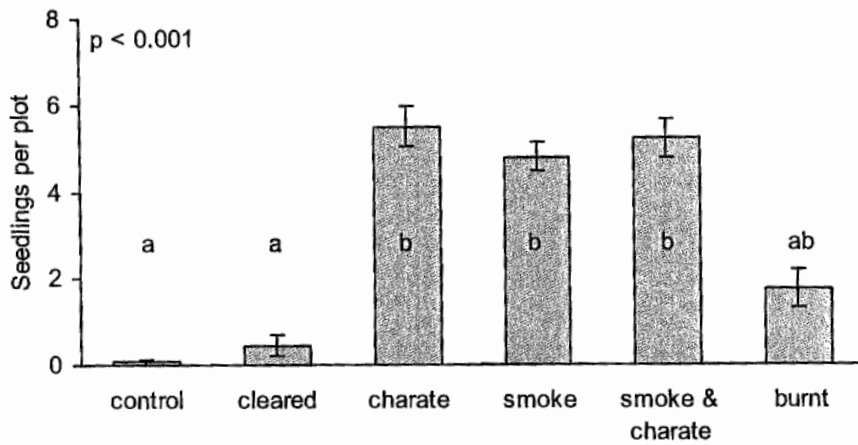
Figure 7.8 Emergence of (a) *Actinotus minor* and (b) *Epacris pulchella* in the field plots; back-transformed data. Error bars are standard error. ANOVA results shown: *P* value given; different letters represent a significant difference (Tukey HSD).
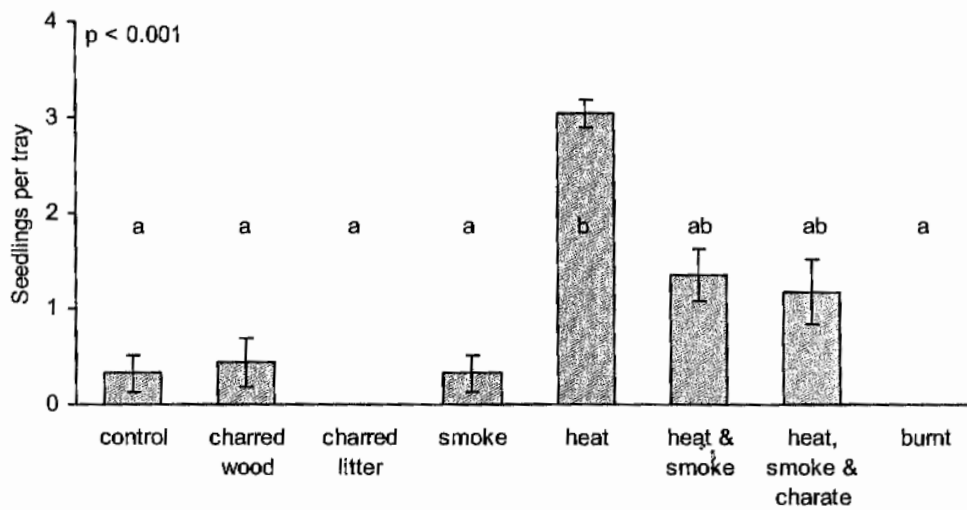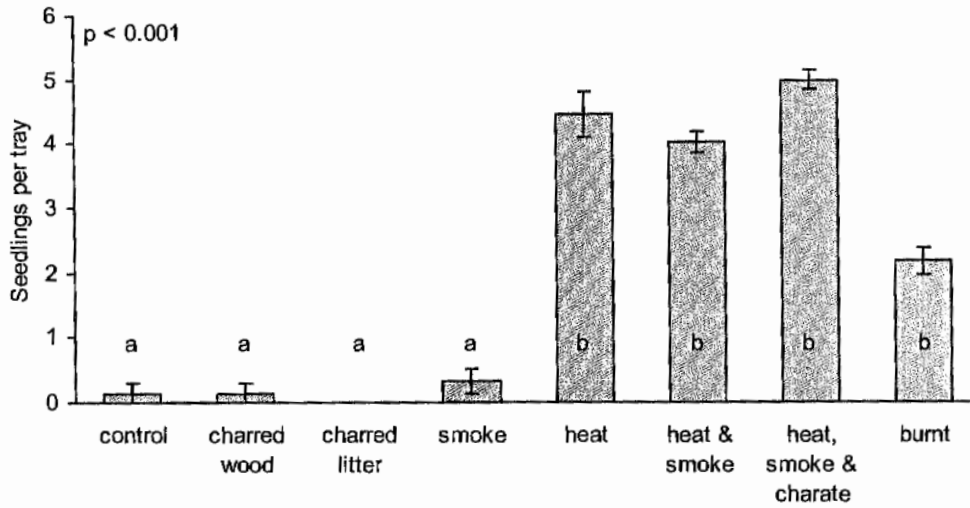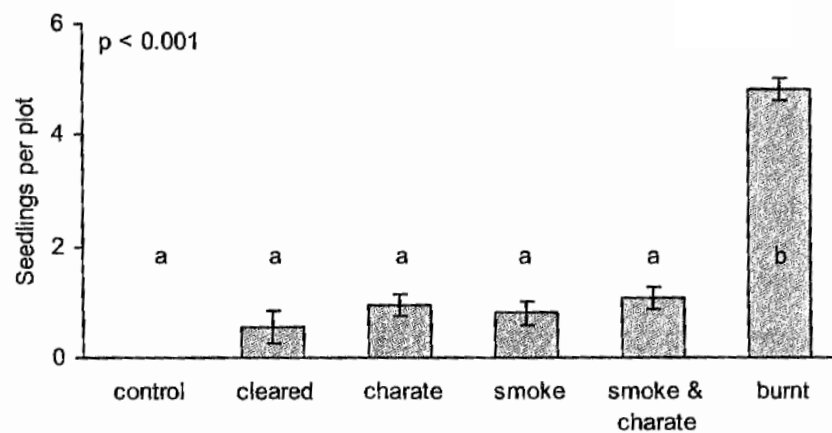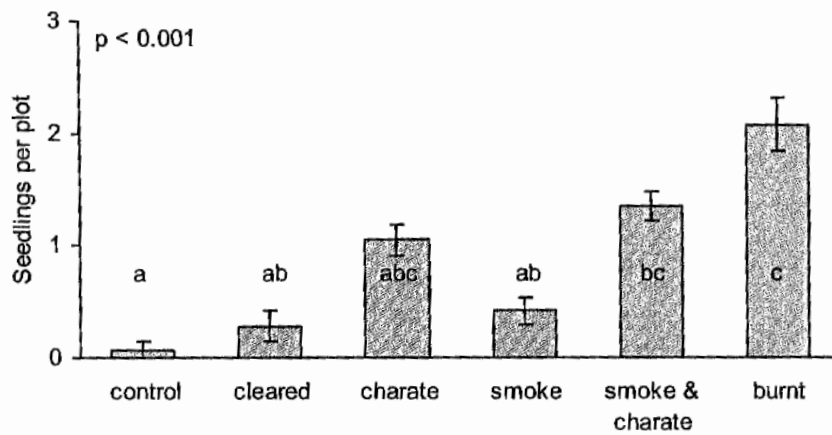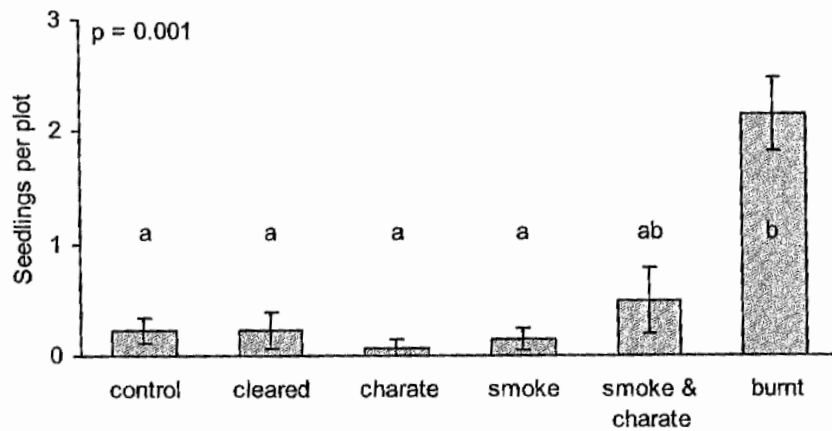
Figure 7.8 continued Emergence of (c) *Micrantheum ericoides*, (d) *Eucalyptus* spp., and (e) Fabaceae species (includes *Acacia myrtifolia, Bossiaea heterophylla, Gompholobium glabratum*, and *Pultenaea* sp.) in the field plots; back-transformed data. Error bars are standard error. ANOVA results shown: *P* value given; different letters represent a significant difference (Tukey HSD).

Discussion

Seedling Density

In the pilot glasshouse trial only the heat-smoke combined treatment significantly increased the density of seedling emergence. This pattern closely reflected that shown by the two most prominent species (*Actinotus minor* and *Epacris pulchella*). It should be noted that dry heating soil in this way is not necessarily an independent treatment as the heating/combustion of organic matter within the soil may provide a similar effect to smoke treatment (Keeley & Nitzberg 1984, Blank & Young 1998, Enright & Kintrup 2001).

Heat alone had no significant effect in this trial. The temperature reached in the soil was not measured, but on reflection may have been inadequate as a heat stimulus (most species respond to a heat cue in the range 60-120 °C; Martin *et al.* 1975, Shea *et al.* 1979, Keeley *et al.* 1981, Auld & O'Connell 1991). Hence, prior to heat treatment in the next trial soil heating was monitored in a sample (Fig. 7.1). This showed that both a higher oven temperature and a longer heating duration than were applied in the pilot trial were required for the soil temperature to reach at least 60 °C.

In the following glasshouse trial, where the temperature reached in the soil was above 60 °C, heat was the most effective treatment. Mean seedling emergence in the heat-treated trays appears higher than that in the burnt soil, but the greater variation made this difference non-significant. Note that the density seen in the burnt soil is underestimated due to the premature germination of some species (*Eucalyptus* spp., Fabaceae spp. and a monocot species). Neither smoke nor charate had a significant effect on soil seedbank seedling emergence, nor was there any interaction between heat and smoke evident as in the pilot trail. Germination of canopy seedbank species was greatest in the charred litter and burnt trays, as most of these came from seeds released by the fire into the burnt sub-site. These species either release seeds sporadically and *en masse* following fire (*Eucalyptus* spp.) or only following the action of fire on serotinous cones (various Proteaceae) (Gill 1981).

In the field plots both smoke and charate improved seedling emergence to the same extent as in the burnt sub-site. This result is curious given the lack of smoke or charate effect seen in the glasshouse trial from the same site. The same method of smoke application was used in these two experiments, the main difference being the watering regime. In the glasshouse, trays were lightly watered following treatment and then subjected to automatic daily watering. In the field, there was light-moderate rainfall at the time of treatment, but overall the rainfall encountered was probably quite low for the first two months of the trial (Fig. 7.2). Little is known about how smoke is leached into the soil profile following fire. Experiments in Chapter 6 indicate that while water may assist the movement of smoke through the substrate it is not essential. It is possible that too much watering in the glasshouse could leach smoke through the depth of soil in the trays before it had enough time to affect most seeds (Grant & Koch 1997).

Given that the smoke and charate effect on seedling emergence was equivalent to that of the fire, heat and combined heat-smoke treatments would have made a very interesting comparison. Unfortunately, it was considered to be too dangerous to apply heat treatment (with a propane torch as in Chapter 6) to the site, as the fuel load of both the litter and shrub layer was quite high (the site was unburnt for 15 years) and the

plots were set up during the fire season (mid-November) at a time when rainfall had been below average (Fig. 7.2).

Variation in the effect of different treatments can be seen between different published seedbank studies in eastern Australia (Table 7.3), some with smoke as the best cue, some with heat best, and some with the two cues equal. It is difficult to determine if these indicate absolute differences in the reaction of communities to heat and smoke cues (potentially with varying proportions of species that respond individually to either heat or smoke or to both) or simply represent variation in treatment methods (temperature and duration of heating, concentration and dilution of smoke).

Few studies have tried to compare the effects on the seedbank of fire-related cues to an actual fire. Roche *et al.* (1998) compared emergence in adjacent burnt (summer) to unburnt smoke-treated (autumn, winter, spring) field plots. Seedling emergence from the smoke-treated plots was highly dependent on the season in which the treatment was applied. Density of native perennial species was unaffected by smoking in spring, and significantly improved by the other treatments in the order winter smoked, burnt, then autumn smoked. Odion (2000) found that seedling emergence was improved by heat, or heat and charate treatments well above the effects of a fire, as the fire caused high levels of mortality among seeds too close to the soil's surface.

Species Richness

The patterns seen in species richness varied a little to the treatment effects on seedling density. In the glasshouse trial, heat was again the dominant treatment, and equivalent to the burnt soil. However, smoke treatment was intermediate between the control and heat treatments. In the field plots, while all treatments again were effective, the effect of the fire was superior.

Vlahos & Bell (1986) predicted that the seedbank of Australian forests would closely resemble the species seen in the standing vegetation, as succession tends to follow the initial floristics model. They didn't find this to be the case, and nor have many other Australian studies (e.g. Ward *et al.* 1997, Morgan 1998, Grant & MacGregor 2001 [Jaccard = 29%], Wills & Read 2002 [Jaccard = 20%, Bray-Curtis = 50%]). The similarity between the standing vegetation and the seedbank seen here was comparatively high at 55%.

Among the species seen only in the seedbank, the two Orchidaceae are cryptic and were possibly overlooked or not visible in the vegetation; and three species (*Allocasuarina* sp., *Cassytha pubescens*, *Solanum* sp.) potentially had seed dispersed into the area from a distance. *Pimelea linifolia* is known to have a shorter adult lifespan than the time since the last fire but a long-lived seedbank, *Acacia myrtifolia* has a moderately short lifespan (and long-lived seedbank), and the *Gonocarpus* sp. is potentially also short-lived (see Table 7.4).

Among the species seen only in the standing vegetation there were species with transient seedbanks, which would only be expected to emerge if sampling coincided with seed release, and species with serotinous seedbanks, which would need to have their seed distribution coincide with the burnt plots or collected charate. This group also included some vertebrate-dispersed species, which possibly had their seed removed from the local area, and ant-dispersed species, which may have had their seeds clustered in

areas or depths that were not sampled. There were also two species with seeds potentially destroyed by fire, and two species with seeds that are potentially very difficult to germinate (see Table 7.4).

Table 7.3 Treatment effects on emergence from the soil seedbank, as reported in the literature. Different letters represent a significant difference, and indicate the level achieved (a lowest to c highest). Seedling density and species richness are recorded separately. n/a means treatment not applied.

Measure	Control	Ash / Charate	Smoke	Heat	Reference
Density	ab	a	bc	c	Enright *et al.* 1997
Richness	a	a	a	a	
Density	a	a	c	b	Enright & Kintrup 2001
Richness	a	a	b	b	
Density	a	n/a	b	b	Read *et al.* 2000
Richness	a	n/a	b	b	
Density	a	n/a	b	c	Wills & Read 2002
Richness	a	n/a	a	b	

Table 7.4 Species found exclusively in either the seedbank or the standing vegetation of the Myall Track prescribed burn site. Attributes are listed that may explain these absences.

Species	Attribute	Reference for attribute
Species found only in the seedbank:		
Acacia myrtifolia	moderately short-lived	T. Auld pers. comm.
Allocasuarina sp.	wind dispersed seed	Benson & McDougall 1995
Cassytha pubescens	vertebrate dispersed seed	Purdie & Slatyer 1976, French & Westoby 1996
Corybas sp.	cryptic	Bishop 1996
Gonocarpus sp.	possibly short-lived	Benson & McDougall 1997, D. Keith pers. comm.
Leptocarpus tenax		
Orchidaceae sp.	cryptic	
Pimelea linifolia	short-lived	Purdie & Slatyer 1976, Clemens & Franklin 1980, D. Keith pers. comm
Platylobium sp.		
Pyllanthus hirtellus		
Solanum sp.	vertebrate dispersed seed	Purdie & Slatyer 1976
Species found only in the standing vegetation:		
Banksia serrata	serotinous seedbank	Blombery & Maloney 1992
Goodenia sp.	germination decreased by fire (seed possibly destroyed)	Purdie & Slatyer 1976
Hakea dactyloides	serotinous seedbank	Blombery & Maloney 1992
Hibbertia sp.	ant dispersed seed	French & Westoby 1996
	difficult to germinate	Chapters 3-5
Lambertia formosa		
Lindsaea linearis	transient seedbank	Benson & McDougall 1993, Keith 1995
Lomandra obliqua	ant dispersed seed	French & Westoby 1996
Lomandra sp.	ant dispersed seed	French & Westoby 1996
	seed possibly destroyed	Chapters 4-5
Monotoca scoparia	vertebrate dispersed seed	French & Westoby 1996
Persoonia levis	vertebrate dispersed seed	French & Westoby 1996
	difficult to germinate	Whitehorne & McIntyre 1975
Xanthorrhoea media	transient seedbank	NPWS 1999
Xyris sp.		

Individual Species

Two species, *Actinotus minor* and *Epacris pulchella*, dominated the soil seedbank of all three experiments (17.2% and 28.5% respectively of the soil seedbank in the pilot glasshouse trial; 16.0% and 44.4% in the Myall Track glasshouse trial; 32.3% and 32.7% in the Myall Track field trial).

Actinotus minor germination appears to be improved by both smoke and heat cues, though the magnitude of the treatment effects varied between the different experiments. In the Myall Track trials the effect of the fire was equivalent to that of smoke or charate alone, and not significantly greater than the control. In the glasshouse trial it was seen that heat-treatment was superior to the effect of the fire. The level of soil heating achieved by the fire is not known, though circumstantial evidence suggests that it was moderate (moderate fuel load pre-fire, good fuel consumption achieved by the fire). The temperature reached may have been less than in the heat treatment, and hence fewer seeds were stimulated to germinate; or it may have been higher, causing some seed mortality. Two Western Australian *Actinotus* species have shown a smoke response (Dixon *et al.* 1995, Roche *et al.* 1997a), one with some interactions between smoke and heat (Tieu *et al.* 2001a).

Germination of *Epacris pulchella* appears to be improved by both smoke and heat, as well as by the fire. Again, variation in treatment effects was seen between the three experiments. However, for *E. pulchella*, the fire was equivalent to smoke and charate treatment in the field and to heat treatment in the glasshouse. At least eight other *Epacris* species have been shown to respond to both smoke and heat, with different interactions when both smoke and heat are combined (Chapter 5, Keith 1997, Gilmour *et al.* 2000, Enright & Kintrup 2001, Wills & Read 2002).

There are many possible explanations for the variation seen in these two species between the three different experiments. There was probably some variation between the effectiveness of the treatments (i.e. ineffective heating in the pilot glasshouse trial, possible smoke leaching in the glasshouse). The environmental conditions (water, light and ambient temperature) encountered in the field are very different to those of the glasshouse. Soil seedbank heterogeneity is also an issue in seedbank studies. While bulking of the soil samples in the glasshouse trials should allow for randomisation of seed occurrence, in the field each treatment replicate can only influence the seeds that naturally occur within that plot. Seedbank distribution is generally quite clustered with respect to adult plant density and location as well as seed dispersal distance (Bigwood & Inouye 1988, Parker *et al.* 1989).

Drosera peltata was also prominent in all three experiments, but no treatment effects were seen. Casual observation indicated a preference for damper soils. Observations also indicated that once the species was established within a tray or plot vegetative spread occurred, thus increasing the density.

Entolasia stricta was seen only in the glasshouse. Germination of this species was significantly greater in the heat treatments and the burnt soil, indicating a heat cue for germination. *Patersonia sericea* was also seen only in the glasshouse. Germination was increased only by heat treatments, indicating a heat cue; however no seedlings were seen in the burnt soil. It is possible that the fire was either unable to stimulate germination, or caused seed mortality (the lethal temperature during a fire depends on the relationship between soil heating and the seed's depth of burial; Auld & O'Connell 1991, Odion 2000). However, there was one unidentified monocot species amongst those that germinated prematurely in the burnt soil.

This may have been *P. sericea*, in which case fire did stimulate its germination, but this went undetected. That seedlings of neither of these two species were seen in the field may be related to inappropriate moisture or temperature regimes for seed germination or seedling survival, or high herbivory levels.

Emergence of *Micrantheum ericoides* in the field was clearly higher only in the burnt sub-site. Smoke and charate did not appear to influence its germination; however seedbank distribution may have had some influence on the results (indicated by a large variance in some treatments). This species was also seen in the glasshouse. While the density was too low for statistical analysis there was a clear dominance in the burnt soil and few seedlings in the other treatments (Table 7.2). This species does appear to be stimulated by fire, but there is no evidence to indicate its preferred germination cue.

In the field plots, density of *Eucalyptus* seedlings was highest in the burnt sub-site where the seed was shed post-fire. Density was also moderate in the charate-treated plots, as the charate source was contaminated with canopy seedbank species. The low occurrence of *Eucalyptus* seedlings in the control and smoke-treated plots indicates that some seed had been released in the unburnt sub-site. While seeds of the dominant tree species are often not encountered in Eucalypt community seedbank studies (Vlahos & Bell 1986, Read *et al.* 2000), this depends on the timing of sampling in relation to seed release (Grant & Koch 1997). Despite sporadic release of *Eucalyptus* seeds from the canopy, no effective soil seedbank accumulates as predation levels are extremely high and seed longevity is short (Ashton 1979). In the glasshouse, density was not high enough for statistical analysis, but seedlings were only prominent in the charred litter treatment (Table 7.2). A few seedlings were seen in the burnt soil trays; however some germination of *Eucalyptus* species occurred prior to placement of soil in the glasshouse.

Fabaceae seedling density in the field was pooled for statistical analysis. Some Fabaceae species occurred in all treatments, indicating that the small non-dormant seed fraction (Auld & O'Connell 1991) required only minor disturbance (e.g. increased ambient soil temperature caused by clearing) to germinate. However, emergence was only significantly improved by fire. Fabaceae species are well known for their heat-stimulated fire response as they possess a water-impermeable hard seed coat (Auld & O'Connell 1991, Bell *et al.* 1993). The density of all Fabaceae species in the glasshouse was too low for statistical analysis. Interestingly there was no apparent heat or fire effect (although several Fabaceae seeds germinated prematurely in the burnt soil, and so were not included in the density counts). It is possible that few Fabaceae seeds were collected within the soil samples taken (small diameter cores of 5 cm depth), as soil seedbank density of hard-seeded legumes is generally quite low (Auld 1986c) and collection of these seeds by ants tends to cluster their spatial distribution and increase their depth of burial (Auld 1986c).

Germinable Soil Seedbank

Estimates of the germinable soil seedbank present at a site will vary depending on the methods used to sample the seedbank (*in situ*, emergence, or extraction methods) and the number and size of sampling units (Roberts 1981, Bigwood & Inouye 1988, Benoit *et al.* 1989). When the emergence method is used, estimates will depend on whether the treatments applied were able to stimulate germination in all species (Roberts 1981, Enright & Kintrup 2001). The potential germinable seedbank of a site can be taken to at least equal the maximum density seen in the most suitable treatment (Enright & Kintrup 2001).

The potential soil seedbank for the community studied (Hawkesbury sandstone woodland, 15 years post-fire) is at least 1291 seeds m^{-2} (Table 7.5). This figure is on par with many other studies of fire-prone woodland communities of eastern Australia (Ata 1992 [cited in Enright & Kintrup 2001], Enright *et al.* 1997, Morris & Wood 2001), but substantially lower than others (Read *et al.* 2000, Enright & Kintrup 2001). Actual seedbank density will vary depending on the seed production and seedbank persistence of the species involved (Thompson & Grime 1979), the successional age of the site (Thompson 1978, Donelan & Thompson 1980) and seasonal heterogeneity (Ward *et al.* 1997); however direct comparisons between studies are difficult given the variability in estimated density caused by different methods and treatments used. There does, however, appear to be a relationship between understorey type and seedbank density in eastern Australian communities, with large seedbanks recorded from areas with grassy understories and/or a high proportion of monocotyledons in the seedbank (Marsden-Smedley *et al.* 1997, Morgan 1998, Read *et al.* 2000, Enright & Kintrup 2001, Grant & MacGregor 2001). The community studied here has a prominently shrubby understorey, and monocotyledons formed a relatively minor part of the seedbank.

The two glasshouse experiments performed here were done on soil from the same general location and vegetation type. Some difference between the seedbank content would be expected as Site 15 (pilot trial) was burnt seven years more recently than Site 16 (Myall Track) and soil was collected in different seasons. The density of seedlings seen in the control treatments was similar for these two sites (Table 7.5), but the potential seedbank density estimates given by these experiments are vastly different (see best treatment; Table 7.5). The difference seen here can at least partly be attributed to the inadequacy of the heat treatment applied in the pilot trial, and illustrates the care that needs to be taken in designing and interpreting seedbank experiments.

The differences in seedling density between the glasshouse and field plots for the same site show how harsh natural conditions are for seedling establishment. The difference presumably reflects both lower germination rates and higher seedling mortality rates in the field compared to the more protective and consistent environment of the glasshouse (Odion 2000).

The extremely low seedling density seen in undisturbed vegetation (i.e. uncleared plots; 'undisturbed' in Table 7.5) shows how dependent this community is on disturbance to stimulate recruitment. While minor disturbance (i.e. clearing of *in situ* vegetation in the field control; soil disruption, increased light and moisture in the glasshouse control) allows some establishment, the vast increase in germination with fire or fire-related treatments demonstrates the community's reliance on fire. The prescribed fire performed resulted in the establishment of 102 soil seedbank seedlings m^{-2} (total establishment 123.2 seedlings m^{-2}), a mere 8% of the potential germinable soil seedbank of the site.

Table 7.5 Soil seedbank density. Only species from the soil seedbank are included (i.e. canopy seedbank and tuber species are excluded). Undisturbed = the uncleared field plots; control = glasshouse untreated soil, cleared but untreated field plots; burnt = soil from the burnt sub-site, field plots in the burnt sub-site; best treatment = applied treatment resulting in highest seedling density (the treatment is given). † Density in the burnt soil in the glasshouse trial is underestimated due to the premature germination of some species.

Trial	Seedlings per m^2 (mean ± standard error)			
	Undisturbed	Control	Burnt	Best treatment
Pilot glasshouse	n/a	72.0 ± 15.0	n/a	264.0 ± 46.2 heat & smoke
Myall Track glasshouse	n/a	84.8 ± 22.3	660.5 ± 43.5†	1290.8 ± 259.5 heat, smoke & charate
Myall Track field plots	2.8 ± 1.2	20.4 ± 6.7	102.0 ± 21.8	118.0 ± 32.7 smoke & charate

CHAPTER 8: GENERAL DISCUSSION

Summary of Results

The germination responses of all species studied have been collated from Chapters 3-7 and are presented in Table 8.1. For each species, responses are summarized as positive, negative or no significant effect with respect to control for each individual treatment studied (charred wood, smoke, heat, and fire). Multiple cue effects are summarized into equal, additive, inhibitory or no combined effect for relevant species. An overall summary is given for whether the species responds to heat, smoke, both heat and smoke, or neither. Seedbank species from Chapter 7 are included in Table 8.1 when the number of seedlings emerging averaged one or more per tray or plot. Species which failed to germinate in any trial are excluded.

A total of 47 species were studied, seven of which failed to germinate in any trial. Ten species were found to be heat responders, five species were smoke responders (heat was inhibitory for three of these), 13 species responded to both heat and smoke, and 11 species showed no significant positive response to any cue (heat was inhibitory for five of these).

The effect of charred wood on species germination was very variable throughout the various experiments performed (Chapters 3, 5-7). Generally, charate had no significant effect on germination in accord with other Australian trials (Bell *et al.* 1987, Marsden-Smedley *et al.* 1997, Enright & Kintrup 2001), though in a few instances it was seen to be either inhibitory or positive. Charate produced in the laboratory by charring stems in a crucible was positive in an initial trial, and had no significant effect in further trials; while that produced in a muffle furnace had no significant effect. Charate collected from the trunks of burnt trees was inhibitory in Petri dish trials, but had no significant effect in glasshouse trials. Charred litter collected in the field post-fire had no significant effect when applied to soil in the glasshouse or where buried seeds were removed from the field after one week, but was positive in the seedbank field plots. This seedbank field trial was the only instance in which charate consistently had a positive influence on germination, and was the situation in which natural conditions were most closely imitated (i.e. charred litter material collected post-fire, left under field conditions for an extended period).

Some of the potential problems with charred wood in germination trials include the effects of different levels of combustion (ash is reported to have inhibitory effects on germination; Neeman *et al.* 1993a, Enright *et al.* 1997, Reyes & Casal 1998) and the variation caused by moisture level (Keeley & Nitzberg 1984). Charred wood therefore seems to be an unreliable germination cue when artificially applied. Given that species with a charate response tend also to show an equal or better response to smoke (Keeley & Bond 1997, Keeley & Fotheringham 1998a), it seems wise to focus on smoke in germination and rehabilitation work.

Table 8.1 Summary of germination results for species studied (species with no germination recorded are excluded). Germination effects of individual charate, smoke, heat and fire treatments: - = not tested; pos = positive; neg = negative; nse = no significant effect of individual treatment. Germination effects of combined heat and smoke treatments: nsd = combined treatment not significantly different to optimal individual treatment; inhibitory = heat treatment counteracted smoke effect; equal = magnitude of germination equal between individual and combined heat and smoke; additive = magnitude of germination improved by combined heat and smoke over individual treatments; unitive = germination only improved above control by combined heat and smoke (no individual treatment effects seen).

Species	Charate	Smoke	Heat	Combined	Fire	Summary
Acacia linifolia	-	nse	neg	-	-	heat inhibitory
Acacia oxycedrus	-	nse	pos	-	-	heat
Acacia suaveolens	nse	nse	pos	nsd	pos	heat
Acacia terminalis	nse	nse	pos	nsd	-	heat
Actinotus minor	pos	pos	pos	additive	nse	heat and smoke
Bauera rubioides	neg	pos	neg	inhibitory	-	smoke (heat inhibitory)
Boronia ledifolia	nse	nse	pos	nsd	-	heat
Calytrix tetragona	nse	pos	nse	nsd	-	smoke
Cassytha pubescens	nse	nse	nse	nsd	-	no effects
Conospermum taxifolium	nse	nse	nse	unitive	-	heat and smoke
Dianella caerulea	nse	nse	nse	nsd	-	no effects
Dianella revoluta	nse	nse	nse	nsd	-	no effects
Dillwynia retorta	nse	nse	pos	nsd	-	heat
Dodonaea triquetra	nse	nse	pos	nsd	-	heat
Doryanthes excelsa	-	-	neg	-	-	heat inhibitory
Drosera peltata	nse	nse	nse	nsd	nse	no effects
Entolasia stricta	nse	nse	pos	nsd	pos	heat
Epacris microphylla var. *microphylla*	neg/nse	pos	neg/pos	equal	-	heat and/or smoke
Epacris pulchella	pos	pos	pos	equal	pos	heat and/or smoke
Eriostemon australasius	nse	nse	nse	unitive	-	heat and smoke
Gahnia sieberiana	nse	nse	nse	additive	-	heat and smoke
Grevillea buxifolia	-	pos	pos	additive	-	heat and smoke
Grevillea sericea	nse	pos	pos	additive	pos	heat and smoke
Grevillea speciosa	nse	pos	pos	additive	pos	heat and smoke
Haemodorum planifolium	nse	nse	neg	nsd	-	heat inhibitory
Kunzea ambigua	nse	pos	pos	equal	-	heat and/or smoke
Kunzea capitata	neg/pos/nse	pos	pos	additive	-	heat and smoke
Lasiopetalum ferrugineum var. *ferrugineum*	nse	nse	pos	nsd	-	heat
Lomandra longifolia	nse	nse	neg	nsd	-	heat inhibitory
Micrantheum ericoides	nse	nse	-	nsd	pos	fire
Mitrasacme polymorpha	nse	nse	nse	unitive	-	heat and smoke
Patersonia glabrata	nse	pos	nse	nsd	-	smoke
Patersonia sericea	nse	nse	pos	nsd	nse	heat
Persoonia pinifolia	nse	nse	nse	nsd	nse	no effects
Phebalium squamulosum subsp. *squamulosum*	nse	nse	nse	nsd	-	no effects
Pimelea linifolia subsp. *linifolia*	nse	pos	neg	inhibitory	-	smoke (heat inhibitory)
Sprengelia incarnata	nse	pos	pos	equal	-	heat and/or smoke
Woollsia pungens	neg	pos	neg	inhibitory	-	smoke (heat inhibitory)
Xanthorrhoea resinifera	-	-	neg	-	-	heat inhibitory
Zieria laevigata	nse	nse	pos	nsd	-	heat

Smoke was found to be a reliable germination cue for many species when applied to individual seeds in the laboratory. Few species, however, responded only to a smoke cue (13% of species studied), which was surprising given how prevalent the smoke response has been reported to be elsewhere (47% of species tested in fynbos; Brown & van Staden 1997; 48 and 54% of species tested in Western Australia; Dixon et al. 1995, Roche et al. 1997a respectively). A large proportion of species studied, however (33%) responded to both smoke and heat, making the total (45%) comparable with other studies. This demonstrates the need for a more holistic approach when studying fire-related germination cues for species from fire-prone floras. The recent concentration on stimulating germination with smoke alone may have over-looked some interesting patterns of multiple germination cues, and failed to find optimal germination stimulation for species in which multiple cues have an additive or synergistic effect.

Seed burial experiments indicated that there is no depth-related variation in the influence of the smoke cues. Indeed, given that the smoke cue is readily water soluble (Chapter 3, de Lange & Boucher 1990, Keeley & Fotheringham 1998a), it is potentially transported through the entire soil profile. For species with a strong smoke response this would leave little opportunity for retaining a residual seedbank post-fire, placing these species in the GI functional type (seedbank exhausted by fire; see Tables 1.4 & 1.5) of Noble & Slatyer (1980).

Application of smoke to seeds stored in soil (either in the glasshouse or field) gave more variable results than did laboratory tests, with the apparent failure of some smoke applications. Given that the smoke cue is water soluble, there appear to be interactions between smoke application and watering regime. In the glasshouse there is the potential to over-water soil, leading to leaching of the smoke cue from the soil before it is able to act on buried seeds. Conversely, under field conditions, a certain amount of rainfall may be required for sufficient transport of the smoke cue. The method of aerosol smoke application is impractical for treating large areas in the field; hence smoke is more often applied in a solution form when utilised for site rehabilitation (Roche et al. 1997b).

With a little fine tuning of the technique, heat was also found to be an easy to apply and reliable cue for collected soil samples, making it an easy way to improve germination of many species in seedbank studies. Application of heat in the field, however is a potentially dangerous activity, and could not easily be used on a large scale for site rehabilitation purposes.

The germination of 25% of the studied species was stimulated by heat alone. Most of these were obligate seeder shrubs with a hard seed coat. Legumes and other hard-seeded taxa are well known for their heat response which breaks the physical dormancy imposed by the seed coat (Bell et al. 1993). Heat treatment has been little studied in non hard-seeded taxa. This study found a substantial number of such species to have their germination stimulated by heat, but most of these also responded to a smoke treatment. In fact, the greatest proportion of studied species (33%) fell into the category of having germination stimulated by both heat and smoke. Among these species there was a fairly even division into species with an equal germination response to both cues, an additive effect when the two cues are applied simultaneously, and a response only when the two cues are applied together.

Ecological Implications

The purpose of a fire-related germination cue is to signal to the buried dormant seed that it is a good time to germinate, as the post-fire environment has many advantages for seedling establishment (e.g. increased resource availability and decreased competition). Since both heat and smoke are signalling the same event, what is the purpose of a species responding to both? This may be a way of being more certain that a fire has occurred, so that germination is more strictly limited to the post-fire environment. For example, heat alone may occur on a hot day in an exposed site (Keeley 1987, Brits et al. 1993, Tieu et al. 2001a; though this is not likely to affect legume species; Auld & Bradstock 1996), while smoke may be transported from a nearby fire (Preston & Baldwin 1999). However, in the event that a fire has actually occurred, a species that reacts to only one of these cues runs the risk that the levels of cue received will be inadequate (e.g. minimal soil heating with low intensity fire; minimal smoke penetration under windy conditions) and hence recruitment will be very low. Being able to respond to either cue reduces this risk. However, if both must be received simultaneously, this risk would be increased.

The species in which germination was stimulated by heat alone were mostly obligate seeder shrubs with a hard seed coat. Among the species studied here the optimal temperature for germination varied from 60-90 °C. Among co-existing species, variation in the level of germination at specific temperatures may help alleviate high levels of seedling competition in the post-fire environment (Trabaud & Oustric 1989). Reliance on a heat cue, however, does mean that post-fire recruitment will be restricted if adequate soil heating is not received (e.g. with low intensity fire; Auld & O'Connell 1991), especially if the temperature range that stimulates germination is narrow. While seeds left dormant by inadequate heat levels will form a residual seedbank to buffer against the effects of a short inter-fire interval, a regime of low-intensity fires presents a threat of local extinction (Auld & O'Connell 1991) as the seedbank slowly declines with minimal replenishment.

The species that responded only to a smoke cue were all from different families, with four shrubs with variable fire response and small seeds and one resprouting herb with rapid post-fire flowering (see Tables 2.1 and 2.3 for species and seed details). For three of these species (*Bauera rubioides*, *Pimelea linifolia* and *Woollsia pungens*), high temperatures had an inhibitory or lethal effect on germination. Given the small size of the seeds of these three species, they are likely to only emerge successfully from burial depths of 2-3 cm (Bond et al. 1999), depths at which inhibitory temperatures are likely to be experienced during moderate or high-intensity fire (Bradstock & Auld 1995). I would therefore predict that these species are more likely to establish seedlings after lower-intensity fires. Casual observation indicates that reliable germination can occur with a moderate to high intensity fire for at least *Pimelea linifolia* (e.g. 1994 wildfire in Ku-ring-gai Chase National Park; personal observation), though no investigation of fire conditions was made. A comparative study of populations of these species under different fire conditions and regimes would be useful.

The potential for smoke to penetrate readily through the soil profile means that there is not the same depth-related germination response shown by heat-responders (Auld & Tozer 1995, T. Auld pers. comm.). This seriously diminishes the ability to retain a residual seedbank post-fire, leaving an obligate

seeder with a strong smoke response vulnerable to local extinction if subject to frequent fire, when inter-fire intervals are less than the time required to build up a new seedbank.

Those species with an equal response to heat and smoke and the two combined were all obligate seeder shrubs with very small seeds. These species had a large non-dormant seed fraction, indicating poor seedbank persistence (Keeley 1991), combined with a strong smoke response leaving little residual seedbank. Without a reliable buffer against frequent fire, it is imperative that these obligate seeders recruit seedlings with every fire event. The ability to germinate with either cue may be a bet-hedging strategy, so that establishment will occur even if one cue is received at an inadequate level (e.g. soil heating too low). I would predict that these species would establish after the greatest range of fire conditions.

Perhaps the most interesting result was those species with an additive response to heat and smoke. While some seeds will germinate if only one cue is supplied, a greater response is elicited when they are combined. Does the action of one cue make the seed more receptive to the action of the other? For example, heat may scarify the seed coat allowing better penetration of smoke into the embryo.

The consequences of this response are similar to the equal response, although maximum recruitment will require both cues simultaneously. These species also had a considerable non-dormant fraction, though on average this was less than those with an equal heat and smoke response. The individual smoke response was also not as strong, thus these species should be left with a greater residual seedbank under less favourable fire conditions. Those species showing this response were all seeders with some resprouting capacity, so that post-fire seedling establishment is important but not always imperative.

Seeds requiring both cues simultaneously (10% of study species) were particularly recalcitrant, with low germination (c. 15%) even with both cues. Fortunately, this limited post-fire recruitment shouldn't lead to local extinction as these species have other traits ensuring their persistence, such as resprouting, post-fire flowering, wide seed dispersal, or long seedbank persistence. *Eriostemon australasius* is of some concern, however while it proves difficult to germinate in the laboratory, it is known to establish well post-fire (Whitehorne & McIntyre 1975), suggesting that it has a germination requirement that hasn't yet been unravelled (for example, a period of soil burial prior to receiving fire-related cues; Tieu *et al.* 2001b).

Table 8.2 summarises the traits common to species in each germination response group. Predictions are made of the effects of particular fire conditions (levels of heat and smoke produced) on persistence (ability to resprout), establishment (potential magnitude of germination), and residual seedbank (influenced by the germination level, depth-related effects of heat vs. smoke, dormancy level, and heat-induced mortality). The subsequent change in abundance of the standing population is estimated (decline, stable, or increase with respect to the pre-fire standing population). A moderate germination level was taken to approximate stand replacement.

The effect of a short inter-fire interval following these fire conditions is also predicted, using the following assumptions. A short inter-fire interval was taken to allow sufficient time for the secondary juvenile period (flowering of resprouting individuals), but not for the average primary juvenile period (flowering of the majority of the new cohort) or fire tolerance (ability of the new cohort to resprout). Thus the effect of a short inter-fire interval is to eliminate the new cohort resulting from the previous

germination event, and stimulate germination of any residual or new (i.e. from resprouting individuals) seedbank. Given the population variability in primary juvenile period (Benson 1985, Ostertag & Meneges 1994), it was assumed that after moderate or high germination levels, some precocious individuals would reach the primary juvenile period before the short inter-fire interval, providing some new available seedbank. Residual seedbank and ability to resprout were taken to buffer the population against frequent fire (Bradstock *et al.* 1998a). Thus extinction risk is maximised by low germination level, no or low residual seedbank, and no or low ability to resprout.

The species potentially at most risk of population decline after an unfavourable fire event are obligate seeders with only one fire-related germination cue. Greatest extinction risk is associated with high heat-producing fires for species with only a smoke response, and low smoke- and heat-producing fires for species with an equal smoke and heat response.

Fires are very variable both at the landscape- and finer-scale. At the landscape scale, fire behaviour varies both spatially (with topography, vegetation structure and fuel load) and temporally (with wind speed and fuel moisture). At a finer scale fire behaviour will vary with factors such as fuel quantity, fuel continuity, fuel curing, wind speed, changes in wind direction, and rock outcrops (Catchpole 2002). Heterogeneity at the fine-scale has been demonstrated in the level of soil heating achieved by a fire (e.g. Atkins & Hobbs 1995, Odion & Davis 2000). This variation has been shown to influence fine-scale patterns in post-fire seedling establishment through both heat-stimulated germination (Bond *et al.* 1990, Hodgkinson 1991) and heat-induce mortality (Borchert & Odion 1995, Odion & Davis 2000). No attempt has been made to measure the spatial variation of the smoke cue produced by a fire. Work here indicates no vertical variation, leaving horizontal variation to have a large influence on fine-scale patterns of germination and residual seedbank for smoke-responding species.

Table 8.2 Traits of species in each germination response group. Consequences of various fire conditions on establishment, residual seedbank, and persistence are predicted. Predictions are also made on: (1) population abundance after one fire event (decline, stable, or increase of standing vegetation cf. pre-fire abundance); and (2) extinction risk of a short inter-fire interval following the given fire conditions (low, moderate, or high extinction probability). See text for details on calculation of extinction risk. Species details and potential germination level are summarised from Chapters 2 and 5.

Germination Response	Traits	Low smoke/low heat	High smoke/low heat	Low smoke/high heat	High smoke/high heat
Heat only	• obligate seeder • hard seed coat • high dormancy • very persistent seedbank • depth-related residual seedbank	• very low germination • high residual seedbank • no resprouting 1 Decline 2 Moderate	• very low germination • high residual seedbank • no resprouting 1 Decline 2 Moderate	• high germination • low residual seedbank • no resprouting 1 Increase 2 Moderate	• high germination • low residual seedbank • no resprouting 1 Increase 2 Moderate
Smoke only	• variable fire response • small seeds • high dormancy • heat sensitive	• very low germination • moderate residual seedbank • moderate resprouting 1 Decline 2 Moderate	• high germination • no residual seedbank • moderate resprouting 1 Increase 2 Low	• very low germination • high mortality • low residual seedbank • low resprouting 1 Decline 2 High	• low germination • high mortality • no residual seedbank • low resprouting 1 Decline 2 High
Smoke & heat equal	• obligate or facultative seeder • small seeds • low dormancy	• low germination • low residual seedbank • moderate or no resprouting 1 Decline 2 High	• high germination • no residual seedbank • moderate or no resprouting 1 Increase 2 Moderate	• high germination • low residual seedbank • no resprouting 1 Increase 2 Moderate	• high germination • no residual seedbank • no resprouting 1 Increase 2 Moderate
Smoke & heat additive	• facultative seeder • moderate dormancy	• low germination • moderate residual seedbank • moderate resprouting 1 Decline 2 Low	• moderate germination • low residual seedbank • moderate resprouting 1 Increase 2 Low	• moderate germination • moderate residual seedbank • no resprouting 1 Stable 2 Moderate	• high germination • no residual seedbank • no resprouting 1 Increase 2 Moderate
Smoke & heat unitive	• resprouter or facultative seeder • high dormancy • seedbank very persistent and/or quickly replenished	• very low germination • high residual seedbank • high resprouting 1 Stable 2 Low	• very low germination • high residual seedbank • high resprouting 1 Stable 2 Low	• very low germination • high residual seedbank • moderate resprouting 1 Decline 2 Low	• moderate germination • moderate residual seedbank • moderate resprouting 1 Increase 2 Low

Management Implications

Both of the prescribed fires studied here stimulated only a small portion of the potential germination. For the individual species studied (*Acacia suaveolens*, *Grevillea sericea*, and *Grevillea speciosa*) the prescribed fire stimulated about 20% germination from each species, when all potentially have germination greater than 80% with more optimal provision of germination cues. In the seedbank examination, the prescribed fire stimulated just over 50% of the potential seedbank to germinate under glasshouse conditions, but only 8% of the potential seedbank emerged under field conditions. In areas where management fires of this type are prescribed on a regular basis, these low levels of establishment will potentially lead to population decline in a wide range of species.

The lack of a depth-related residual seedbank for species with a strong smoke response has serious implications for the survival of these species under frequent fire regimes. An obligate seeder with no residual seedbank will become locally extinct if inter-fire intervals are less than the time taken to build a new seedbank. This phenomenon has been well demonstrated for obligate seeders with serotinous seedbanks (e.g. Bradstock & O'Connell 1988), where the entire canopy-stored seedbank is exhausted (released to germinate or destroyed) by a single fire event (Bradstock & Myerscough 1981). Species with soil-stored seedbanks have been assumed to be more resilient to extremes of fire frequency, due to the buffering effect of a residual seedbank. This assumption may need to be re-evaluated for species demonstrated to have a strong germination response to smoke.

The variety of responses to heat-shock treatment seen here suggest that the proportion of different species germinating after a fire will vary markedly with the level of soil heating achieved. While many of the species studied require temperatures in the range 60-90 °C to stimulate germination, others are inhibited or killed at temperatures of 80-120 °C. These effects have been observed post-fire, with legume species germinating more prolifically after high-intensity fires (Christenson & Kimber 1975, Auld 1986b, Auld & O'Connell 1991). On the other hand, heat-sensitive species germinate only or more prolifically after lower-intensity fire (Moreno & Oechel 1991, Tyler 1995).

That species with different germination requirements and hence different reactions to fire intensity and frequency can co-exist demonstrates that these elements of the fire regime have been variable enough at a landscape scale in the past to maintain a species balance. Future land management must ensure that this variability is continued in order to maintain biodiversity. While the effects of fire frequency have been shown to have a greater floristic influence than the effects of fire intensity (Bond & van Wilgen 1996, Morrison 2002), the results here (Table 8.2) suggest that further research into the demographic effects of different fire conditions on species with different fire-related germination cues would be valuable.

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