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Dormancy release in Australian fire ephemeral seeds during burial increases germination response to smoke water or heat

Katherine S. Baker[1]*, Kathryn J. Steadman[1], Julie A. Plummer[1], David J. Merritt[2] and Kingsley W. Dixon[2]

[1]School of Plant Biology, Faculty of Natural and Agricultural Sciences, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia; [2]Botanic Gardens and Parks Authority, Kings Park and Botanic Garden, West Perth, WA 6005, Australia

Abstract

Fire ephemerals are short-lived plants that primarily germinate after fire. Fresh and laboratory-stored seeds are difficult to germinate *ex situ*, even in response to fire-related cues such as heat and smoke. Seeds of eight Australian fire ephemeral species were buried in unburnt and recently burnt sites of natural bushland during autumn. Seeds were exhumed after 6 and 12 months and incubated in water and smoke water, either with or without a heat treatment at 70°C for 1 h. Generally, germination did not increase after 6 months of burial, but after 12 months of burial germination was enhanced in seven of the eight species. *Actinotus leucocephalus* produced higher germination following 12 months of burial without any further treatment, and smoke water and heat further improved germination. The four *Gyrostemonaceae* species, *Codonocarpus cotinifolius*, *Gyrostemon racemiger*, *Gyrostemon ramulosus* and *Tersonia cyathiflora*, only germinated in the presence of smoke water, and their germination was enhanced by burial. Burial improved germination in response to a heat treatment in *Grevillea scapigera* and *Alyogyne huegelii* seeds, but did not enhance *Alyogyne hakeifolia* germination. During concurrent dry laboratory storage of seeds at 15°C, only *Actinotus leucocephalus* produced increased germination in response to smoke water and heat over time. In summary, soil burial can alter the dormancy status of a number of Australian fire ephemeral seeds, rendering them more responsive to germination cues such as smoke water and heat. The requirement for a period of burial before seeds become responsive to smoke and/or heat would ensure that seeds persist in the soil until a subsequent fire, when there is an increase in nutrients available for growth and reduced competition from other plants.

Keywords: dormancy release, fire ephemeral, germination, *Gyrostemonaceae*, heat, smoke water, soil burial

*Correspondence:
Fax: +61 8 6488 1186
Email: Katherine.Baker@nt.gov.au

Introduction

Fire ephemerals are a functional group of plants that are found in many fire-prone regions of the world, such as California, South Africa and Australia (le Maitre and Midgley, 1992). These species form transient components of the above-ground flora, usually only germinating after fire, growing rapidly, flowering, producing seed and dying before a subsequent fire (Bell *et al.*, 1984; Hunter, 1998). Monocarpic fire ephemerals flower and fruit once, and usually live for less than 1 year, whereas polycarpic fire ephemerals flower and fruit for more than one season and may live for up to 10 years (Pate *et al.*, 1985). After the plants die, these species exist only as seeds in the soil seedbank.

Australian fire ephemerals germinate from the soil seedbank in large numbers after fire in natural bushland (Bell *et al.*, 1984). However, germination of fresh or laboratory-stored seeds is difficult. Many viable fire ephemeral seeds that are fresh or laboratory-stored, fail to germinate following incubation at a range of temperatures or treatment with fire-related cues such as heat, smoke, nitrate and ethylene (Parsons, 1997; Hunter, 1998; Baker *et al.*, 2005). Even setting fire to hay over fresh or laboratory-stored fire ephemeral seeds placed in the soil does not induce germination (Baker, unpublished results). It has been inferred that under natural conditions, seeds of fire ephemerals persist in the soil seedbank for long periods between fires (Pate and Hopper, 1993; Egerton-Warburton, 1998). Thus, low germination of laboratory-stored

seeds, compared to the profuse emergence of these species from the soil seedbank after bushfires, suggests that some form of pre-fire conditioning is required.

A period of soil burial alters the conditions necessary for germination in a number of species. Some species produce higher germination after a period of burial (Kilian and Cowling, 1992; Pickup *et al.*, 2003), and others change in their sensitivity to germination stimulants, such as light, nitrate (Wesson and Wareing, 1967; Derkx and Karssen, 1993) and smoke (Doherty and Cohn, 2000). Germination of fire-following species has also been enhanced by a period of burial. In South Africa, more seeds from *Audouinia capitata* fruits germinated in response to smoke after a period of burial than when freshly collected (de Lange and Boucher, 1993). In California, three fire-following species, which did not germinate when initially treated with heat, smoke, nitrate and ethylene, germinated in response to smoke following a year of burial (Keeley and Fotheringham, 1998). Likewise, a number of Western Australian species produced higher seedling emergence in response to aerosol smoke following a period of burial (Roche *et al.*, 1997; Tieu *et al.*, 2001). Many of these studies did not separate the effects of ageing from burial. However, Tieu *et al.* (2001) examined concurrently the effects of laboratory storage and found that some species required burial, whereas others just required a period of dry storage. Roche *et al.* (1997) investigated the effect of storing seeds in soil punnets in a glasshouse, whereas Tieu *et al.* (2001) buried seeds in bushland to better reflect natural conditions of soil burial. Both studies measured changes in germination response as seedling emergence from soil punnets. However, generally low seedling emergence in these studies may have underestimated actual germination.

Here, we investigated whether the germination of eight fire ephemerals from the south-western botanical province of Western Australia was influenced by 6 or 12 months of soil burial in natural bushland, in unburnt and recently burnt soil. It was hypothesized that recently burnt soil would be more conducive to overcoming dormancy than unburnt soil. The requirement for further treatments, such as smoke and heat following burial, was also examined. To separate any effects of burial and ageing, germination tests were also undertaken on seeds concurrently stored under laboratory conditions. In contrast to those of Roche *et al.* (1997) and Tieu *et al.* (2001), these tests were undertaken in Petri dishes rather than soil punnets, to measure germination rather than emergence. A glasshouse trial was also established to determine whether glasshouse soil storage mimicked burial in the field. The effect of soil burial under natural conditions has not been examined previously in any of these species. A better understanding of the requirements for dormancy breaking and germination stimulation in these species will ensure greater efficiency in the use of these seeds in land rehabilitation and conservation programmes.

Materials and methods

The germination responses of eight Western Australian fire ephemeral species from the *Apiaceae*, *Gyrostemonaceae*, *Malvaceae* and *Proteaceae* families were examined. All species were polycarpic fire ephemerals, except for *Actinotus leucocephalus*, which is a monocarpic fire ephemeral. Seeds were either collected during summer 2001–02 or purchased from commercial seed suppliers (Table 1). Prior to use all

Table 1. Seed collection date and provenance for the species studied

Species	Family	Collection date	Source
Actinotus leucocephalus Benth.	*Apiaceae*	12/2001	Collected from burnt site 40 km N Gin Gin, WA, 31°01.231′S, 115°43.297′E
Alyogyne hakeifolia (Giord.) Alef.	*Malvaceae*	12/1999	Purchased from Nindethana Collection from Salmon Gums, WA
Alyogyne huegelii (Endl.) Fryxell	*Malvaceae*	12/1999	Purchased from Nindethana Collection from near Esperance, WA
Grevillea scapigera A.S. George	*Proteaceae*	12/2001	Received from Kings Park, WA Collection from a translocated population at Corrigin, WA
Codonocarpus cotinifolius (Desf.) F. Muell.	*Gyrostemonaceae*	1/11/1997	Purchased from Nindethana Collection from Hyden, WA
Gyrostemon racemiger H. Walter	*Gyrostemonaceae*	21/12/2001	Collected from burnt site 55 km N Gin Gin, WA 30°54.384′S, 115°38.417′E
Gyrostemon ramulosus Desf.	*Gyrostemonaceae*	Pre-1989	Purchased from Nindethana Collection from Carnarvon, WA
Tersonia cyathiflora (Fenzl) J.W. Green	*Gyrostemonaceae*	6/1/2002	Collected from burnt site Beekeepers Reserve, North of Eneabba, WA 29°32.315′S, 115°03.415′E

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seeds were cleaned, air dried and stored in sealed containers at 15°C. Seeds obtained from Nindethana Seed Service had been stored at 4°C prior to purchase in February 2002.

Field burial trial

A burial trial was undertaken in an area of *Banksia attenuata* woodland on Karrakatta sands in the Spearwood dune system (McArthur, 1959) at The University of Western Australia's Shenton Park Field Station, Perth. Part of the bushland had been burnt in January 2002. Rainfall and temperature data for the duration of the trial were obtained from the nearby Department of Agriculture Floreat Park weather station (Fig. 1).

Seeds were buried in nylon mesh bags in March 2002 (autumn) before the onset of the rainy season (Fig. 1) at four random sites within each of the burnt and unburnt areas of bushland. Seeds were buried in both burnt and unburnt sites to determine whether post-fire soil conditions were more conducive to alleviating dormancy. The finely woven mesh bags allowed the transfer of water and solutes, but contained the seeds. For each species, 130 seeds which appeared healthy and intact, were placed in each bag, together with an approximately equal volume of fine white sand. At each of the eight sites, the surface 2 cm of soil was removed from a 2 m^2 area, and then the underlying 3 cm of soil was removed separately. Ten free-draining trays were positioned in the hole and the lower 3 cm of soil placed in the trays. A seed bag of each species was placed in each tray and covered with the surface 2 cm of soil. Bird netting was stretched over each site to reduce seed predation.

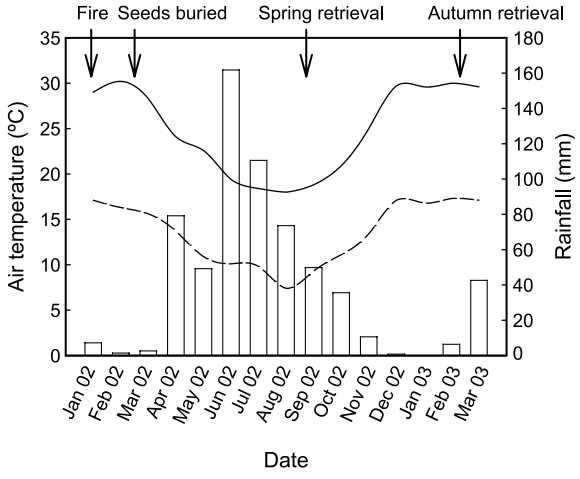

Figure 1. Mean monthly minimum (dashed line) and maximum (solid line) air temperature (°C) and monthly rainfall (mm, white bars) between January 2002 and March 2003.

Half of the trays at each of the four plots within the burnt and unburnt sites were exhumed 6 months after burial, in late September 2002 (spring) as air temperatures started to rise and rainfall decreased, and the remainder were exhumed 1 year after burial, in March 2003 (autumn) at the end of the summer dry season. At each exhumation the number of seedlings that had germinated in the plots was noted. The moisture content (% fresh weight) was determined for the seeds retrieved from burial in spring, at the end of the winter wet season. Exhumed seeds were sealed in plastic bags and, within 24 h of collection, a subsample of each species (10–80 seeds, depending on seed size) from each of the plots within each of the burnt and unburnt sites was weighed, placed in an oven for 17 h at 105°C, and reweighed (International Seed Testing Association, 1999). Seeds exhumed in spring were allowed to air dry in the laboratory for 1 week prior to germination testing. For all exhumed seeds, damaged, flat or germinated seeds were counted and discarded. For each of the eight plots (four replicates in each of the burnt and unburnt sites), 25 healthy seeds of each species were assigned to control, smoke water, heat and combined heat and smoke water treatments. Seeds assigned to either the heat, or heat and smoke water treatments, were wrapped in aluminium foil and placed in an oven at 70°C for 1 h and then cooled at 20°C for 24 h. To minimize fungal contamination, all seeds were surface sterilized prior to incubation by shaking for 30 s in 2% (v/v) NaOCl containing a non-ionic surfactant (one drop per 125 ml of Plus 50, Ciba-Geigy, Sydney). Seeds in NaOCl were then placed under vacuum for 5 min, returned to normal air pressure for 5 min and placed under vacuum again for 5 min to remove air pockets around the seeds. Seeds were then transferred to a laminar flow hood, and using sterile, distilled water, rinsed three times, then soaked for 10 min before a further rinsing. Seeds assigned to smoke water treatments were air dried in the laminar flow hood for 8–12 h before transfer to Petri dishes, to ensure imbibition of the smoke water.

Seeds were placed on two pieces of Whatman No. 1 filter paper over three pieces of 4 cm^2 absorbent sponge in sterile plastic Petri dishes (90 mm diameter). Petri dishes were moistened with either 10 ml of sterile distilled water or a 1:10 dilution of smoke water. Both solutions contained 0.15% (v/v) Previcur fungicide (Aventis, Melbourne, active ingredient 600 $g\,l^{-1}$ propamocarb). Smoke water was prepared by filtering (0.2 μM) Seed Starter (Kings Park and Botanic Garden, Perth) produced according to Tieu *et al.* (1999), except that hay was burnt instead of native vegetation. Aliquots of Seed Starter were frozen until required, to minimize any possible changes over time. Petri dishes were sealed with Parafilm to retain moisture. Seeds

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were incubated under a daily 12 h light, 12 h dark regime at a constant 20°C. Light was provided by cool white fluorescent tubes [50 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR)]. Seeds were monitored weekly for 5 weeks, and seeds were removed as they germinated. Seeds were considered to have germinated once the radicle emerged >2 mm. Remaining seeds were dissected to determine whether they were filled. Results are presented as a percentage of filled seeds.

The same germination tests (water, smoke water, heat, heat and smoke water) were undertaken on seeds stored at 15°C in the laboratory at the time seeds were buried (autumn 2002) and when seeds were exhumed for the second time (autumn 2003), to determine whether any differences in germination of buried seeds were due to the storage environment or age. At the time of the first seed exhumation (spring 2002), all four germination tests were undertaken on laboratory-stored *Actinotus leucocephalus* and *Codonocarpus cotinifolius* seeds, and the remaining species were germination tested in water only.

Shadehouse burial trial

To determine whether field conditions could be replicated under nursery conditions, the effect of soil storage of seeds in a shadehouse was also investigated. At the end of autumn (March) 2002, laboratory-stored seeds of all eight species were placed in seedling punnets (13 × 8 × 4.5 cm deep) containing a pasteurized mix of sand, composted sawdust and peat (1:1:1), as per Roche *et al.* (1997) and Tieu *et al.* (2001). Seeds were covered with a fine layer of sand, watered and placed in a tin shed. Smoke, produced by hay burnt in a metal drum, was fan-forced through a pipe into the shed. Control punnets were set up in a similar way, but not smoked. Punnets were transferred to a shadehouse at The University of Western Australia, Perth and watered daily until spring (September) 2002. During the summer the punnets remained dry, except during a few showers (Fig. 1). In autumn (March) 2003 the smoked punnets were re-smoked, following the same procedure. Watering to all punnets then recommenced and continued until spring (September) 2003. Seedling emergence was monitored weekly during periods of watering.

Statistics

One-way analyses of variance (ANOVAs) were undertaken to determine whether the percentage of filled seeds changed over time when seeds were buried in each of the burnt and unburnt sites, and to compare germination within each species over time under controlled laboratory conditions and after burial in the burnt and unburnt plots. Where germination was absent from all four replicates, the treatment was excluded from analysis, to satisfy the ANOVA assumption of equal variances. *t*-tests were used to compare the percentages of filled seeds and germination levels after 6 and 12 months between the burnt and unburnt sites. A two-way ANOVA was performed to determine whether seed moisture content was similar between the burnt and unburnt plots. Percentage data were arcsine square-root transformed prior to analysis. Fisher's protected LSD test was used as a post-hoc test. Treatments were regarded as significantly different if $P < 0.05$. Statistical analyses were performed in Genstat version 6 (VSN International, Oxford, UK).

Results

Field burial trial

During 12 months of soil burial, many of the species experienced a decline in the number of filled seeds, calculated as the proportion of seeds filled out of the percentage of seeds retrieved from burial that were undamaged and firm (Fig. 2). Four species, *Alyogyne hakeifolia*, *Alyogyne huegelii*, *Codonocarpus cotinifolius* and *Gyrostemon ramulosus*, exhibited large declines (44–84%) in the proportion of filled seeds during the first 6 months of burial (Fig. 2B, C, E, G), which coincided with the wet winter season (Fig. 1). Although the two *Alyogyne* species displayed similar declines in filled seeds between the burnt and unburnt sites, greater declines in the proportion of filled seeds occurred in the burnt sites for *C. cotinifolius* and *G. ramulosus*. *Gyrostemon racemiger* and *Tersonia cyathiflora*, which are in the same family as *C. cotinifolius* and *G. ramulosus*, did not experience any reduction in the proportion of filled seeds during 12 months of burial (Fig. 2F, H). The remaining species, *Actinotus leucocephalus* and *Grevillea scapigera*, exhibited a small decline (18–33%) in filled seeds over the 12 months of burial (Fig. 2A, D). In spring, some seedlings, mainly *A. leucocephalus*, *A. hakeifolia* and *A. huegelii*, were observed in the burnt plots, representing <0.6% of seeds buried at that site. No seedlings of the study species were observed in either the unburnt plots or the surrounding area. No seedlings were observed in autumn in either the burnt or unburnt plots.

Prior to burial, germination of many species was low (Figs 3, 4). Only *A. hakeifolia* and *A. huegelii* seeds germinated in water, but germination of neither species exceeded 30% (Fig. 3E, I). Smoke water led to some germination of pre-buried *A. leucocephalus* and *C. cotinifolius* seeds, but germination was still below 40% (Figs 3B, 4B). Heat enhanced germination of *A. leucocephalus* and *A. huegelii*, resulting in 27% and 45%

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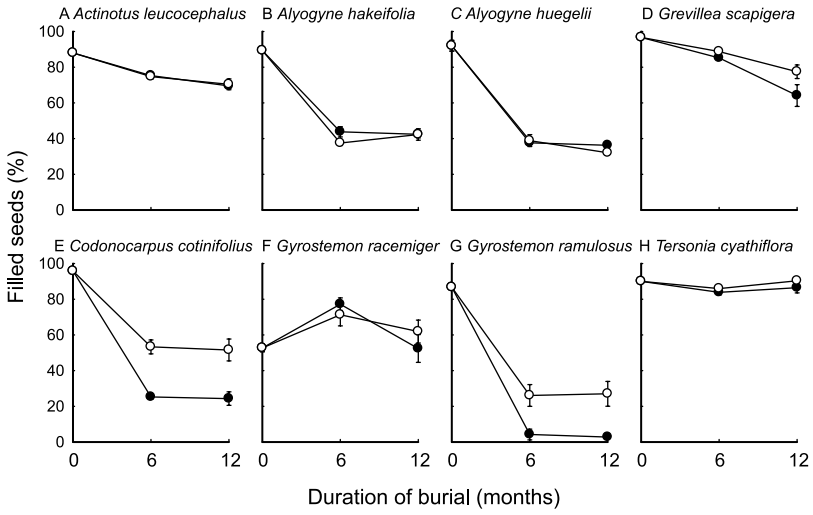

Figure 2. Filled seeds (mean ± SE) at the time of burial and after 6 and 12 months of burial in burnt (●) and unburnt (○) soil for each species. (A) *Actinotus leucocephalus*; (B) *Alyogyne hakeifolia*; (C) *Alyogyne huegelii*; (D) *Grevillea scapigera*; (E) *Codonocarpus cotinifolius*; (F) *Gyrostemon racemiger*; (G) *Gyrostemon ramulosus*; and (H) *Tersonia cyathiflora*.

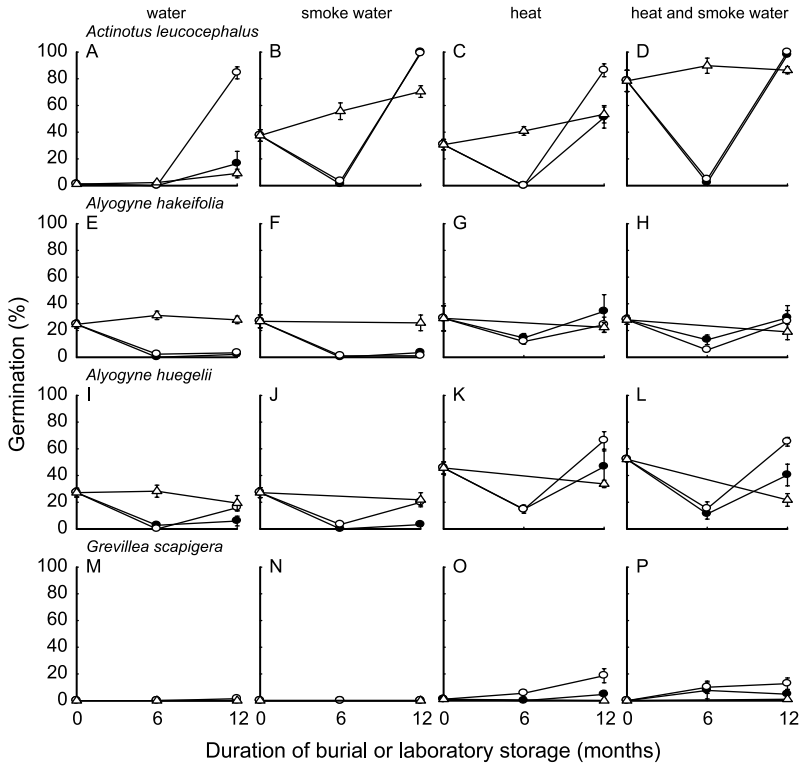

Figure 3. Germination (mean ± SE) as a percentage of filled seeds for (A–D) *Actinotus leucocephalus*; (E–H) *Alyogyne hakeifolia*; (I–L) *Alyogyne huegelii*; and (M–P) *Grevillea scapigera*, when treated with water (A, E, I, M), smoke water (B, F, J, N), heat (C, G, K, O) and heat and smoke water (D, H, L, P). Seeds were stored in the laboratory at a constant 15°C (△), or buried in burnt (●) or unburnt (○) soil for 6 and 12 months.

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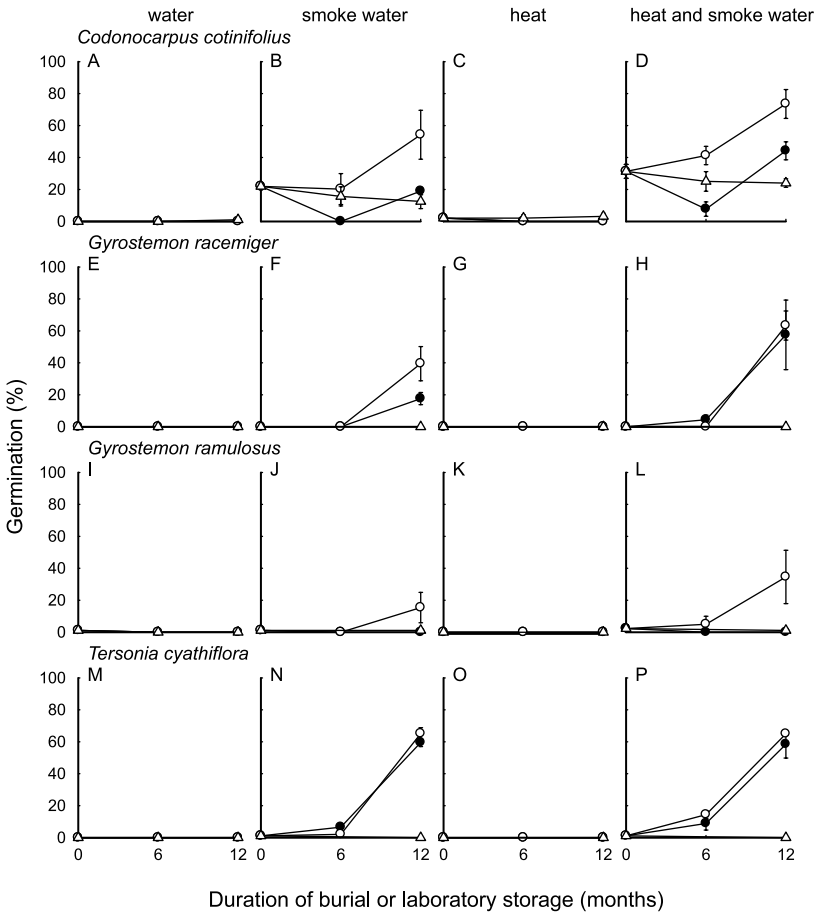

Figure 4. Germination (mean ± SE) as a percentage of filled seeds for the four *Gyrostemonaceae* species. (A–D) *Codonocarpus cotinifolius*; (E–H) *Gyrostemon racemiger*; (I–L) *Gyrostemon ramulosus*; and (M–P) *Tersonia cyathiflora* when treated with water (A, E, I, M), smoke water (B, F, J, N), heat (C, G, K, O) and heat and smoke water (D, H, L, P). Seeds were stored in the laboratory at a constant 15°C (△), or buried in burnt (●) or unburnt (○) soil for 6 and 12 months.

germination, respectively (Fig. 3C, K). Combining the heat and smoke water treatments produced higher germination than either treatment individually for *A. leucocephalus* (78% germination, Fig. 3D) and *C. cotinifolius* (31% germination, Fig. 4D). Four species, *G. scapigera*, *G. racemiger*, *G. ramulosus* and *T. cyanthiflora*, produced negligible germination prior to burial irrespective of incubation in water or smoke water, or whether they were heat treated (Figs 3M–P, 4E–P).

Germination was influenced by the duration of burial. Six months of burial during the winter wet season failed to enhance germination of most species, and actually suppressed germination of those species that had germinated before burial (Figs 3, 4). For example, prior to burial *A. leucocephalus* produced up to 78% germination in the heat and smoke water treatments, but after 6 months of burial neither heat nor smoke water induced any germination in this species (Fig. 3B–D). In contrast, *G. scapigera* and *T. cyathiflora*, which did not germinate prior to burial, produced low levels of germination (<15%) after burial for 6 months; *G. scapigera* responded to heat and *T. cyathiflora* responded to smoke water (Figs 3O, 4N).

Following a further 6 months of burial over the hot, dry summer (Fig. 1), germination of most species was enhanced above pre-burial levels (Figs 3, 4). *A. leucocephalus* was the only species that produced higher germination in water after 12 months of burial (Fig. 3A). Most other species required either smoke water and/or heat treatments following burial to increase germination above pre-burial levels. *A. leucocephalus* germination was also enhanced further by the smoke water and heat treatments (Fig. 3A–D). Heat treatments produced higher germination in the two *Alyogyne* species after 1 year of burial, compared to non-heat treated seeds (Fig. 3E–L). All four *Gyrostemonaceae* species exhibited an increase in germination following burial for 12 months, but only when incubated in smoke water (Fig. 4). The only species that produced lower

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germination after 12 months of burial than before burial were unheated *A. hakeifolia* seeds and *A. huegelii* seeds exhumed from the burnt plot (Fig. 3E, F, I, J). For both *Alyogyne* species, any filled seeds remaining at the conclusion of the germination tests, regardless of treatment, were impermeable to water.

Generally, germination was either similar between seeds exhumed from the burnt and unburnt sites, or higher in the latter. For example, germination of *T. cyathiflora* was similar following burial in the burnt and unburnt sites (Fig. 4M–P), while *C. cotinifolius* seeds produced higher germination in smoke water after burial in the unburnt site than the burnt site (Fig. 4B). In *A. leucocephalus*, germination in water was higher after seeds were buried for 12 months in the unburnt site than the burnt site (Fig. 3A), but after a heat treatment this difference was diminished (Fig. 3A, C). Similarly, smoke water enhanced more *G. racemiger* seeds to germinate after 12 months of burial in the unburnt site than the burnt site, but heating the seeds prior to smoke water treatment resulted in similar levels of germination between the two sites (Fig. 4F, H). The moisture content of seeds exhumed in spring varied between species (8–27%), but was consistently higher in those seeds exhumed from the burnt site than the unburnt site (Fig. 5).

Germination of seeds stored in a controlled temperature room at 15°C exhibited different patterns to those buried in the field (Figs 3, 4). Laboratory-stored seeds did not exhibit the increase in germination noted for many of the seeds after 12 months of burial, nor suppression of seed germination observed after 6 months of burial. In fact, germination of most laboratory-stored seeds remained unchanged throughout the year. The only species to exhibit a change in germination response during the year were *A. leucocephalus* and *A. huegelii*. Germination of *A. leucocephalus* increased over time when seeds were incubated in smoke water or heated (Fig. 3B, C), whereas *A. huegelii* germination declined when heated and incubated in smoke water (Fig. 3L).

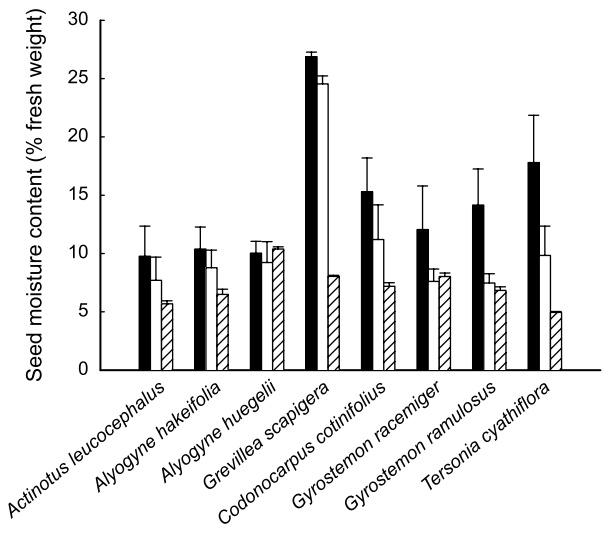

Figure 5. Seed moisture content (% fresh weight, mean ± SE) of each species following exhumation in spring 2002 from the burnt (filled bars) and unburnt (open bars) sites, and after air dry storage in the laboratory at 15°C (bars with diagonal lines).

Shadehouse burial trial

Fresh or laboratory-stored seeds placed in soil and either aerosol-smoked for an hour or left unsmoked

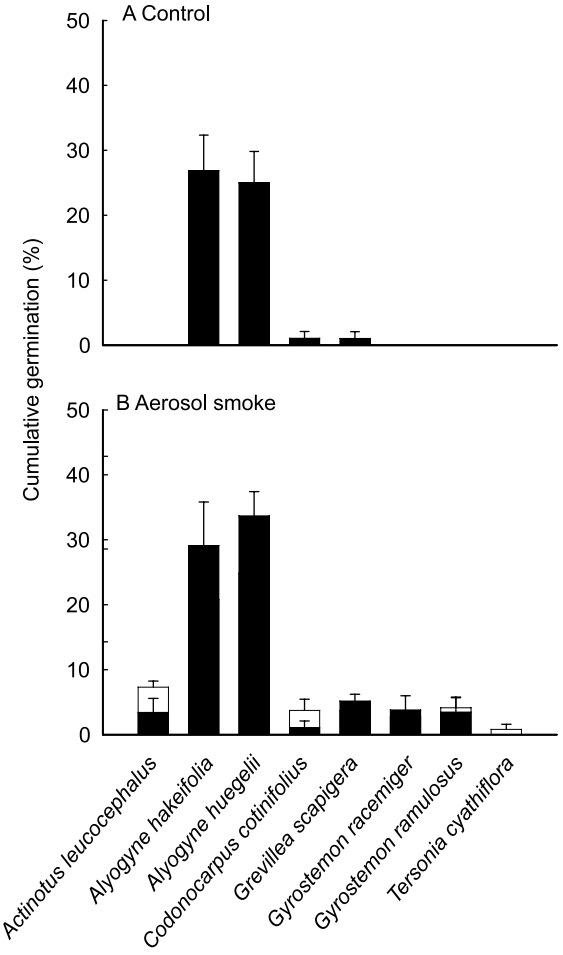

Figure 6. Germination (mean ± SE) of the eight species when (A) placed in soil and (B) given an aerosol smoke treatment. Aerosol smoke treatments were performed in autumn (March) 2002 and autumn (March) 2003. Filled bars indicate germination during the first winter season and the open bars represent germination over the second winter in the soil. Germination is expressed as a percentage of filled seeds buried at the start of the experiment.

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generally produced low levels of emergence (≤5%; Fig. 6). However, moderate numbers (25–35%) of *A. hakeifolia* and *A. huegelii* seedlings emerged in the first year, with no difference in emergence between the smoked and unsmoked punnets. Emergence of unsmoked *A. leucocephalus*, *G. scapigera*, *G. racemiger* and *G. ramulosus* seedlings was negligible (≤1%), and only slightly higher (3–5%) when aerosol-smoked. Some *A. leucocephalus* and *C. cotinifolius* seeds emerged after an aerosol-smoke treatment in the second year, but only to low levels (7 and 4%, respectively).

Discussion

Seeds of fire ephemerals are typically exposed to a period of soil burial, because these species predominantly germinate after fire, release their seeds into the soil seedbank and adult plants usually die before a subsequent fire (Pate *et al.*, 1985). Burial influenced the subsequent germination of the eight species examined. Germination of five of the six species with water-permeable seeds and physiological dormancy (Baker *et al.*, 2005) was enhanced in smoke water by 12 months of burial. Thus, in these species, physiological dormancy was alleviated during burial, allowing them to respond to germination stimulants such as smoke (Vleeshouwers *et al.*, 1995).

Fire ephemerals characteristically germinate after fire, grow rapidly and reach reproductive maturity quickly (Bell *et al.*, 1984; Pate *et al.*, 1985; Hunter, 1998). Consequently, seeds may be dispersed into an environment with some residual smoke cues (Roche *et al.*, 1998), but without the other conditions optimal for growth of these highly plastic fire ephemerals, such as the flush of nutrients released post-fire and reduced competition from established plants (Bell *et al.*, 1984; Pate *et al.*, 1985). A requirement for a period of seed burial before seeds become responsive to smoke would ensure that seeds persist in the soil until a subsequent fire rather than germinate in a suboptimal environment. These findings also highlight that other species from fire-prone regions, which produce negligible or low germination in response to smoke water when freshly collected or after laboratory storage (Pierce *et al.*, 1995; Hunter, 1998), may not necessarily lack a smoke response once dormancy is alleviated.

The only monocarpic species examined, *A. leucocephalus*, differed from the remaining species, which were polycarpic fire ephemerals, in that it after-ripened during 12 months of laboratory storage and produced high germination percentages after burial without any further heat or smoke cues. Pate and Hopper (1993) suggested that monocarpic fire ephemerals have shorter-lived seedbanks than polycarpic fire ephemerals, and perhaps the characteristics displayed by *A. leucocephalus* may indicate greater ease of dormancy release and the ability to germinate in the absence of fire. These features would increase the likelihood of species survival if the fire interval exceeded seed longevity. However, more monocarpic fire ephemerals need to be examined before general conclusions about monocarpic versus polycarpic fire ephemerals can be drawn.

The duration and/or seasonality of burial influenced germination. Six months of winter burial did not enhance germination in any of the species, and actually suppressed germination of some species that germinated prior to burial, such as *A. leucocephalus*. However, following subsequent summer burial, germination of all but one species was higher than after 6 months of burial. This may suggest the presence of dormancy cycling in response to the seasons, and the presence of dormancy cycling would be consistent with the presence of non-deep physiological dormancy in many of these species (Baskin and Baskin, 2004; Baker *et al.*, 2005). Dormancy cycling is exhibited by numerous species (Derkx and Karssen, 1993; Auld *et al.*, 2000; Benech-Arnold *et al.*, 2000; Tieu *et al.*, 2001), and may be present in other species with soil-stored seeds in south-western Australia, as seedling emergence is higher after field sites are smoked in autumn, than in spring (Roche *et al.*, 1998). Similarly, seed germination from soil-stored *Audouinia capitata* fruit in South Africa, which also has a mediterranean climate, varies in response to smoke according to season (de Lange and Boucher, 1993). Few seedlings that germinate in spring survive the subsequent summer drought (Roche *et al.*, 1998), highlighting possible evolutionary selection for seeds that germinate in autumn in environments with winter dominant rainfall.

Alternatively, observed germination may have been lower after 6 months of winter burial if seeds had already germinated while buried. For example, as some *C. cotinifolius* seeds were smoke responsive prior to burial, they may have germinated in the burnt site when smoke chemicals leached through the soil with the onset of winter rain. Indeed, fewer filled *C. cotinifolius* seeds were retrieved from the burnt site than the unburnt site, and seeds from the burnt site exhibited a reduced response to smoke water. For *A. hakeifolia* and *A. huegelii*, which have physical, and possibly also physiological, dormancy (Baker *et al.*, 2005), the proportion of filled seeds declined during the first 6 months of burial and all remaining filled seeds were impermeable. This suggests that the permeable fraction of the seed lots (approximately 50%; Baker *et al.*, 2005) either germinated or decayed, and winter conditions did not alleviate physical dormancy in the remaining seeds.

Seed persistence in the soil is important for species, such as fire ephemerals, that require disturbance for recruitment and where the plants live for shorter periods than the average disturbance interval

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(Auld *et al.*, 2000; Holmes and Newton, 2004). As in other species in the hard-seeded *Fabaceae*, *Geraniaceae* and *Malvaceae* families, the impermeable fractions of the *A. hakeifolia* and *A. huegelii* seedlots are likely to persist in the soil for many years (Halloin, 1983; Holmes and Newton, 2004). However, seed persistence in the soil is not limited to species with impermeable seed coats (Thompson *et al.*, 2003). *G. racemiger* and *T. cyathiflora* exhibited no reduction in the proportion of filled seeds during 12 months of burial, suggesting that seeds of these species could persist in the soil for many years. However, *G. ramulosus* and *C. cotinifolius* experienced large reductions in the proportion of filled seeds during 12 months of burial, possibly because these seeds were older when buried.

When germination of exhumed seeds differed between the burnt and unburnt sites, it was generally higher following burial in the latter, contrary to expectations. In the process of burying seeds, the ground cover was removed from both sites. However, there was greater regrowth on the burnt site, primarily of the weed *Ehrharta calycina*, which may have shaded the soil and reduced temperatures. Seed moisture content was higher in the burnt site than the unburnt site at the end of winter, suggesting that moisture levels differed between the two sites. Hence, temperature and soil moisture may have differed between the sites, which would have influenced the depth of physiological seed dormancy (Vleeshouwers *et al.*, 1995; Benech-Arnold *et al.*, 2000).

Germination following burial in the field and incubation in the laboratory was generally higher than emergence after soil-storage in the shadehouse. Emergence in the shadehouse was similar to that obtained by Roche *et al.* (1997) for species that required a period of burial before seeds responded to smoke. Lower germination in the shadehouse may have resulted from different conditions between punnet and field soil storage. Differences in soil temperatures and moisture contents between the shadehouse and field would be anticipated, due to differences in the amount and timing of water application (daily sprinkler versus rain), and differences in solar radiation, atmospheric humidity and soil type (Businger, 1966; Bachelard, 1985). Alternatively, seeds in the shadehouse may have germinated, but seedlings did not emerge above the soil surface and went undetected. Regardless, field burial and germination under controlled conditions provided a greater estimate of the proportion of seeds that were responsive to smoke.

Germination of three of the species was not greatly enhanced by burial or post-burial treatment. *A. hakeifolia* germination was not enhanced by burial, and increases in *A. huegelii* and *G. scapigera* germination were less than 20%. Although the 70°C for 1 h heat treatment induced some germination of *A. huegelii* seeds after 12 months of burial, ungerminated seeds retained physical dormancy. Heating seeds to 100°C for 1 h overcomes physical dormancy in these *Alyogyne* species, but reduces viability (Baker *et al.*, 2005), suggesting that an intermediate heat treatment may be more appropriate, such as 100°C for 5 min, which promotes the germination of a number of chaparral shrubs (Keeley, 1987). *G. scapigera* germination was variable, with low germination resulting from heat treatments after field burial, or aerosol smoke after shadehouse storage. A higher response to aerosol smoke was observed by Roche *et al.* (1997), but there was large variability between replicates. This variability may suggest that this species requires specific soil temperature and moisture requirements for dormancy release during burial, which might reflect the narrow distribution of this species (Rossetto *et al.*, 1995). Burial of *G. scapigera* seeds in the Corrigin region, where this species occurs (drier, hotter and with lower and less seasonal rainfall than Perth; Australian Bureau of Meteorology, 1975), might result in higher subsequent germination in response to heat and smoke water than after burial in Perth.

In summary, the germination of a number of Australian fire ephemerals can be enhanced by smoke water or heat treatments following 12 months of soil burial in natural bushland and exhumation in autumn. Six months of burial and exhumation in spring either did not enhance, or suppressed, germination, indicating that seeds were undergoing seasonal dormancy cycling or required a longer duration of burial to enhance germination. Previously, many of these species were considered difficult to germinate. Improved understanding of the requirement for burial prior to smoke exposure in many of these seeds will ensure more effective and efficient use of seeds, which has potential cost benefits for horticultural and land rehabilitation industries, and conservation benefits for rare species. These findings highlight that conditions of seed storage can alter seed dormancy, and hence, influence their response to stimulants such as smoke water. Further investigations are required to confirm whether temperature and soil moisture are the factors altering the dormancy states of these seeds during burial, enabling these seeds to respond to fire-related cues.

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