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Interaction of soil burial and smoke on germination patterns in seeds of selected Australian native plants

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Abstract

Patterns of dormancy and smoke responsiveness in artificially buried seeds were investigated in eight native plant species from the species-rich mediterranean-type climate zone of south-western Australia. A comparison was made between germination and viability behaviour of shelf- and field-soil-stored seed, with and without smoke treatment, at least every 3 months. These comparisons corresponded with each of the four seasons. The species chosen germinated with the aid of smoke under field or glasshouse conditions (termed 'dormant'; $n = 4$) or produced low and variable germination under glasshouse conditions with smoke (termed 'deeply dormant'; $n = 4$). Three trends were observed in viability of soil-stored seeds over 450 d: no decline, gradual decline or late-onset decline. In addition, various germination responses to soil burial and aerosol smoke were observed. Burial was not required for optimal germination in *Anigozanthos manglesii.* However, for all other species tested, maximum germination was observed only following a period of burial. This was manifested in a germination response without smoke after a short period of burial (*Stylidium affine* and *Conospermum triplinervium*) or a longer period of burial (*Conostylis neocymosa*, *Hibbertia commutata*, *Leucopogon conostephioides*, *Stirlingia latifolia* and *Stylidium crossocephalum*). Smoke treatment led to high germination in buried seed of *S. affine*, *S. crossocephalum* and *H. commutata*. The patterns of germination detected in this limited number of species indicate that a variety of mechanisms may exist, both temporally and spatially, in south-western Australian species, and support further research of this type for horticulture and land restoration.

Keywords: burial, smoke, germination, *Anigozanthos manglesii*, *Conostylis neocymosa*, *Stylidium affine*, *Stylidium crossocephalum*, *Hibbertia commutata*, *Leucopogon conostephioides*, *Conospermum triplinervium*, *Stirlingia latifolia*

Introduction

Germination cycles of soil-buried seeds closely correlate with season(s) conducive to optimal germination and seasonal conditions that enforce dormancy in seeds of annual species (Karssen, 1980/81; Baskin and Baskin, 1994, 1995, 1998; Egley, 1995). Mass germination of soil-borne seeds after a disturbance usually associated with a fire episode (Went *et al*., 1952; Keeley, 1991; Bell *et al*., 1993; Dixon *et al*., 1995) further supports the role of soil–seed interaction coupled with smoke and/or fire for many species. Therefore it is possible that burial in soil causes physical and/or physiological changes in seeds that allow them to respond to germination signals (smoke) that would be ineffective after a similar period of conventional dry storage (van Staden *et al*., 1994; Roche *et al*., 1997; Keeley and Fotheringham, 1998).

Species (70% of 180 Western Australian species; Roche *et al*., 1997) which have initiated the after-ripening process (>3 months old) and germinate with smoke treatment are termed 'dormant' species. The remaining species, which do not germinate regardless of the dry after-ripening treatment of the seeds and smoke treatment, are termed 'deeply dormant'. Seeds of deeply dormant species appear to require soil burial for maximal germination, as seeds that were soil stored for 1 year and then smoke treated produced significantly higher germination results than those stored in laboratory conditions prior to exposure to smoke (Roche *et al*., 1997).

The current study tests the hypothesis that soil burial modifies seeds of deeply dormant species to become more responsive to the smoke cue, while not

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modifying dormant seeds in this way. Unlike the study of Roche *et al*. (1997), where seeds were stored in punnets of soil exposed to seasonal changes in a glasshouse, the seeds in the current study were buried in natural field conditions. The outcomes of the study are important for devising methods to improve seed germination for species from the diverse ecosystems of south-western Australia.

Materials and methods

Test species

The eight indigenous species from south-western Australia selected for this study (Table 1) were from fire-prone flora and, apart from *Anigozanthos manglesii*, have a strong requirement in nature for fire or disturbance to elicit large-scale germination (Dixon *et al*., 1995). Seeds of *A. manglesii* and *Stirlingia latifolia* were collected from a *Banksia* woodland (Gnangara, 30 km NE of Perth 31°47′S 115°51′E) while *Conostylis neocymosa*, *Stylidium crossocephalum*, *Conospermum triplinervium* and *Leucopogon conostephioides* were collected from a kwongan heathland (Eneabba, 300 km N of Perth 29°5′S 115°1′E). *Stylidium affine* and *Hibbertia commutata* were collected from a jarrah (*Eucalyptus marginata*) forest, 300 km S of Perth (33°4′S 115°0′E). All species, with the exception of *C. triplinervium*, *L. conostephioides* and *S. latifolia*, consisted of one embryo, endosperm and non-multiplicative seed coat. Seeds of *L. conostephioides* are contained in drupaceous woody fruits with up to five locules containing embryos, while *C. triplinervium* and *S. latifolia* possess embryonic seeds (Corner, 1976) consisting of an outer seed covering and embryo. Seeds were collected during November to December 1996. The seeds were air-dried (30 ± 5°C) (mean ± SD) for 2 weeks, cleaned and stored in air-tight jars at room temperature (21 ± 2°C).

Seed storage methods

Buried seeds were encased in nylon mesh (0.1 mm) sachets (5 × 5 cm) containing acid-washed and dried silica sand. Control seeds were stored at room temperature (22 ± 2°C) in envelopes in a light-excluding cupboard. The number of replicates of sachets, each containing approximately 200 seeds, was dependent on available seed quantity. Three replicate sachets were allocated to *S. affine*, *H. commutata*, *L. conostephioides*, *S. latifolia* and *C. triplinervium*, four to *C. neocymosa* and *S. crossocephalum*, and five to *A. manglesii* for each retrieval time. Seeds collected from Eneabba were buried in soils in open kwongan heath at Eneabba, 300 km north of Perth, and those collected from other locations were buried in soils in *Banksia* woodland at Gnangara, 30 km north-east of Perth. Sachets of seeds were prepared so that there was sufficient material for seven burial and retrieval cycles at Eneabba and nine retrieval cycles at Gnangara. All seeds were buried in December 1996. Sachets were placed 10 cm apart and at 2 cm depth in soil. Replicate sachets were retrieved monthly for species buried at Eneabba and fortnightly for species buried at Gnangara during the first summer (January to March 1997). Sachets were later retrieved from both locations at the end of the four seasons (12 months). Seed burial times approximated the expected periods of seed dehiscence under natural conditions.

Soil temperature and moisture of study sites

Data loggers (Tiny Tag^{TM}) programmed to record temperature every 2 hours were buried adjacent to the seed sachets (2 cm) at Eneabba and Gnangara. Data were converted to monthly mean minima and maxima. Soil moisture was determined at each retrieval time by the gravimetric method, after Hillel (1971); 300 g of soil sampled from five sites chosen at random were weighed before and after being oven

Table 1. List of dormant and deeply dormant study species

Dormancy type	Family	Species
Group 1 Dormant	*Haemodoraceae*	*Anigozanthos manglesii* D. Don
	Haemodoraceae	*Conostylis neocymosa* Hopper
	Stylidiaceae	*Stylidium affine* Sonder
	Stylidiaceae	*Stylidium crossocephalum* F. Muell.
Group 2 Deeply dormant	*Proteaceae*	*Conospermum triplinervium* R. Br.
	Dilleniaceae	*Hibbertia commutata* Steudel
	Epacridaceae	*Leucopogon conostephioides* DC.
	Proteaceae	*Stirlingia latifolia* R. Br.

dried at 105°C for 24 h. Soil moisture was calculated as:

$$\% \text{ moisture} = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}}.$$

The mean and standard error was calculated for each sampling time.

Viability tests

The viability of shelf-stored and buried seeds retrieved during 15 months was determined by a cut test. Viable seed was determined by dissection of the endosperm and presence of a turgid, white and intact endosperm in three replicates each of 50 seeds or fruits, and expressed as a percentage of seeds tested. Similar measures have been used for estimating viability in other Australian species (Paynter and Dixon, 1990; Lamont and Runciman, 1993; Schatral and Fox, 1994; Roche *et al*., 1997).

Germination tests

At each sampling time, contents of sachets were pooled, after which lots of 50 seeds per replicate (three replicates of *S. affine*, *H. commutata*, *L. conostephioides*, *S. latifolia* and *C. triplinervium*, four of *C. neocymosa* and *S. crossocephalum* and five of *A. manglesii*) were allocated to control and smoke treatments. An equal number of replicates and seeds of control, shelf-stored seeds were included. For each species (Table 1), shelf-stored, soil-stored, smoke-treated shelf-stored and smoke-treated soil-stored seeds were sown in a 1:1:1 mixture of peat:aged sawdust:sand in punnets and covered with 2–3 mm of silica sand. Punnets were watered thoroughly after seeds were sown. Punnets requiring smoke treatment were aerosol smoked for 1 h (Roche *et al*., 1997). This involved smoke fumigation of punnets of sown seeds in a steel-framed plastic tent (2 m × 1.5 m × 1 m). Smoke was produced in a 20-litre metal drum by slow combustion of a mix of fresh and dry vegetation from native *Banksia–Eucalyptus* woodland in Perth. Smoke produced in the drum was drawn by a small pump through a 1.5 m galvanized pipe and 2 m of plastic hosing into the tent. Punnets were incubated at 18 ± 2°C (mean ± SD), under a photoperiod of 10 h:14 h (light:darkness) to emulate autumn germination conditions. As seedling emergence was slow, germinants were removed and scored fortnightly for up to 12 weeks. As a representative measure of germination, all germination results were adjusted for viability determined at the time of retrieval using the approach of Roche *et al.* (1997). Here we have defined germination percentage of viable seeds as 'viability adjusted germination' (VAG) as determined by:

$$\text{VAG} = \frac{\text{Germination (\%)}}{\text{Viability}} \times 100.$$

Statistical analyses

For each species, one-way analysis of variance (ANOVA) was undertaken on viability and VAG data, followed by Fisher's least significant difference (LSD) and using $P < 0.05$ as the criterion for significance of effect. Datasets were checked for normality and, where appropriate, were arcsine transformed prior to ANOVA. Retransformed data are presented in the results.

Results

Soil temperature and moisture

At Eneabba (Fig. 1a), the mean temperature in the warmest month (January 1997) was 57.3°C, with an absolute maximum temperature of 62.0°C. At Gnangara (Fig. 1b), summer temperatures were lower, with a mean maximum temperature of 52.4°C and an absolute maximum temperature of 60.1°C. Soil moisture peaked at 3–5% between June and September, with a minimum of less than 1% between November to March at both sites.

Changes in seed viability

Seed viability of shelf-stored *S. latifolia* was significantly reduced after day 45 and remained constant to 450 d ($P < 0.05$) (Table 2). For the remaining species, there was no significant decline in viability of shelf-stored seed after 15 months ($P > 0.05$). In addition, viability decline of soil-stored seeds for 15 months varied between the species and did not correlate with dormancy type or family (Tables 1 and 2). Three trends in viability change of soil-stored seeds were observed in the eight species tested (Table 2):

1. A progressive decline in viability upon burial in *C. triplinervium*, *L. conostephioides*, *S. latifolia* and *S. crossocephalum* and after the first summer in *A. manglesii*.
2. A delay in decline in viability was found in *C. neocymosa* and *H. commutata* (Table 2).
3. No loss of viability after 15 months in *S. affine* (Table 2).

Release from dormancy and germination in all species was not necessarily correlated to viability decline. There was little correlation between changes in viability found during periods of soil storage and seed dormancy type (dormant or deeply dormant) in all species tested. Decline in viability of buried seeds

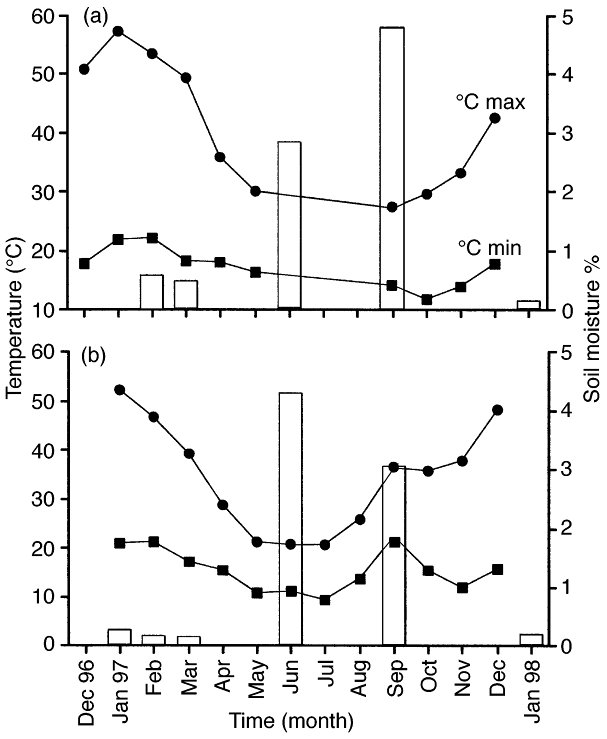

Figure 1. Mean maximum and minimum soil temperatures (°C) and soil moisture (%) 2 cm below ground level at (a) Eneabba and (b) Gnangara in the south-west of Western Australia. The top line (●) represents the mean maximum temperature, the bottom line (■) represents mean minimum temperature and the bars represent soil moisture. Soil moisture in April, May, July, August, October to December 1997 and soil temperature at Eneabba between June and August 1997 were not measured.

was not consistent in genera (*S. affine* and *S. crossocephalum*) nor in seed type (i.e. *C. neocymosa* and *S. affine*, which maintained high viability until the onset of the second summer, are very different in size, shape and seed coat structure).

Requirement for smoke, soil storage and seasonal influences on seed germination

Four germination types were observed.

1. Gradual dormancy relief

Dormancy release in *A. manglesii* progressed with time, becoming more smoke responsive over 450 d, with greatest germination following a second summer regardless of storage type (Table 3).

2. First summer dormancy relief

Very low (<5%) germination of non-smoked seeds, either shelf- or soil-stored, occurred within 450 d for *S. affine* (Table 3). Following the first summer (100 d), germination of smoked seeds was high (up to 50%) for soil-stored seeds, and 20% for shelf-stored seeds. Germination was low for the remainder of the year. Smoke significantly ($P < 0.05$) improved germination over non-smoked seeds for both shelf- and soil-stored seeds of *S. affine* only after 75 d. Summer burial (100 d) alone increased germination of *C. triplinervium,* although only to a low level. Germination of *C. triplinervium* seeds buried for 100 d was not increased by smoke treatment.

3. Bimodal dormancy relief

Both unsmoked shelf- and soil-stored seeds of *C. neocymosa* and *S. crossocephalum* germinated most prolifically after summer (100 d and 450 d) ($P < 0.05$) (Table 3). There was an unexpectedly high germination (>80%) event in shelf-stored and smoke-treated seed of *C. neocymosa* at 270 d, which was uncharacteristic of the general trend of germination for other species. With this exception, germination was significantly higher ($P < 0.05$) after 450 d burial (after two summers) over shelf storage for the same period in *C. neocymosa* and *S. crossocephalum*. Germination of smoke-treated shelf- and soil-stored *C. neocymosa* and *S. crossocephalum* was significantly higher ($P < 0.05$) than unsmoked treatments (except for soil-stored *C. neocymosa* at 100 d) within the first summer.

4. Strong soil-storage requirement

Seeds of *H. commutata*, *L. conostephioides* and *S. latifolia* did not germinate following the first 100 d during soil burial over summer, or did so in low numbers, after which time a small, yet significant ($P < 0.05$) number of smoke-treated seeds germinated (<30%) at 450 d (Table 3). Unlike *H. commutata*, where germination of smoke-treated, soil-stored seed for 360 d was significantly ($P < 0.05$) higher (5.6%) than unsmoked (2.1%) seed, there were no significant differences ($P > 0.05$) between smoke-treated shelf-stored and buried seeds in *L. conostephioides* (Table 3). Buried *S. latifolia* seed germinated at significantly higher levels after 450 d, regardless of the presence or absence of the smoke treatment.

Discussion

This study indicated a degree of intra-generic and intra-familial variation in dormancy release and germination strategies in seeds of some Western Australian plant species, providing a potential mosaic of germination opportunities. This was illustrated, first, by the finding that soil burial modified the dormancy status of some deeply dormant species as well as some dormant species. Secondly, we noted distinct patterns of dormancy release and

Table 2. Viability of eight Western Australian species following shelf storage and burial for up to 450 days

Dormancy type and species	Days Season	30 Summer	45	60	75	100 Autumn	180 Winter	270 Spring	360 Summer	450 Autumn
Dormant										
Anigozanthos manglesii[1]	Shelf	98.7 (0.7)^{de}	98.7 (0.7)^{de}	99.3 (0.7)^{de}	100 (0)^{e}	99.3 (0.7)^{de}	100 (0)^{e}	99.3 (0.7)^{de}	100 (0)^{e}	99.3 (0.7)^{de}
	Buried	97.3 (0.7)^{e}	98.7 (0.7)^{de}	98.7 (0.7)^{de}	100 (0)^{e}	99.3 (0.7)^{de}	88.7 (0.7)^{c}	79.3 (3.7)^{de}	72.0 (4.0)^{ab}	62.0 (3.1)^{a}
Conostylis neocymosa[2]	Shelf	96.7 (1.7)^{bc}		100 (0)^{e}		98.7 (0.7)^{cde}	100 (0)^{e}	99.3 (0.7)^{de}	100 (0)^{e}	100 (0)^{e}
	Buried	96.7 (0.7)^{b}		98.7 (0.7)^{b}		97.3 (0.7)^{bc}	98.7 (0.7)^{cde}	98 (0)^{bcd}	98 (0)^{bcd}	79.3 (5.3)^{a}
Stylidium affine[1]	Shelf	95.3 (1.3)^{a}	95.3 (1.3)^{a}	97.3 (1.8)^{a}	98.7 (0.7)^{ab}	98 (1.1)^{ab}	100 (0)^{b}	98.7 (0.7)^{ab}	98.7 (0.7)^{ab}	98.7 (1.3)^{b}
	Buried	96.7 (0.7)^{a}	98.7 (0.7)^{ab}	95.3 (2.7)^{a}	98.7 (0.7)^{ab}	99.3 (0.7)^{b}	96.0 (1.1)^{a}	98.0 (2.0)^{b}	96.0 (1.1)^{a}	100 (0)^{b}
Stylidium crossocephalum[2]	Shelf	96.7 (0.7)^{def}		*		96.7 (2.4)^{f}	94.0 (1.1)^{de}	96.7 (1.3)^{ef}	95.3 (1.3)^{def}	94.7 (0.7)^{def}
	Buried	90.7 (1.8)^{de}		*		90.0 (3.1)^{c}	85.3 (1.8)^{d}	71.3 (6.8)^{b}	72.0 (1.1)^{b}	42.0 (4.2)^{a}
Deeply dormant										
Conospermum triplinervium[2]	Shelf	62.0 (3.1)^{de}		*		30.0 (3.1)^{de}	33.3 (5.2)^{de}	26.0 (1.1)^{de}	24.7 (2.4)^{d}	26.0 (2.0)^{de}
	Buried	32.0 (1.1)^{de}		*		18.7 (0.7)^{c}	15.3 (4.4)^{c}	5.3 (0.7)^{b}	4.0 (1.1)^{b}	0 (0)^{a}
Hibbertia commutata[2]	Shelf	100 (0)^{bc}		100 (0)^{c}		100 (0)^{c}	100 (0)^{c}	100 (0)^{c}	100 (0)^{c}	
	Buried	98 (1.1)^{bc}		96.7 (1.3)^{cb}		99.3 (0.7)^{bc}	99.3 (0.7)^{bc}	99.3 (0.7)^{bc}	94.7 (0.7)^{a}	
Leucopogon conostephioides[2]	Shelf	89.3 (2.4)^{c}		*		*	*	66.0 (5.8)^{c}	75.3 (5.9)^{c}	62.7 (2.9)^{c}
	Buried	65.3 (6.4)^{c}		*		*	*	47.3 (0.7)^{b}	28.0 (4.6)^{a}	28.0 (1.1)^{a}
Stirlingia latifolia[1]	Shelf	36.7 (0.7)^{e}	25.3 (5.8)^{d}	16.7 (1.7)^{cd}	*	18.7 (2.4)^{cd}	22.0 (2.3)^{cd}	24.0 (1.1)^{d}	19.3 (1.7)^{cd}	21.3 (3.7)^{cd}
	Buried	46.7 (4.8)^{e}	22.0 (2.0)^{cd}	14.0 (2.0)^{bc}	*	14.0 (2.0)^{bc}	4.0 (1.1)^{ab}	8.7 (3.3)^{ab}	7.3 (0.7)^{ab}	4.0 (2.0)^{ab}

Data are mean percentage viability (± SE) and values followed by the same letter(s) are not significantly different ($P < 0.05$).
*Omitted due to inadequate seed numbers.
[1] Species sampled four times over the summer season.
[2] Species sampled twice over the summer season.

Table 3. Viability adjusted germination (VAG) of eight Western Australian species following shelf storage and burial for up to 450 days with and without the addition of smoke treatment

Dormancy type and species	Days Season	30 Summer	45	60	75	100 Autumn	180 Winter	270 Spring	360 Summer	450 Autumn
Dormant										
Anigozanthos manglesii^{1}	Shelf	0 (±0)^{a}	0.8 (±0.5)^{abcd}	0 (±0)^{a}	0.4 (±0.4)^{ab}	1.6 (±0.7)^{bcde}	2.4 (±0.7)^{def}	0 (±0)^{a}	0.8 (±0.5)^{abcd}	2.4 (±1.1)^{cde}
	Shelf+smoke	0.8 (±0.8)^{abc}	2.0 (±0.9)^{bcde}	3.2 (±0.8)^{ef}	5.2 (±1.8)^{f}	4.8 (±1.7)^{f}	8.8 (±0.5)^{gh}	10.4 (±1.2)^{h}	31.2 (±4.3)^{j}	21.2 (±4.6.)^{i}
	Buried	0 (±0)^{a}	0 (±0)^{a}	0.4 (±0.4)^{ab}	0 (±0)^{a}	1.6 (±0.7)^{bcde}	0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}	4.8 (±0.8)^{fg}
	Buried+smoke	0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}	4.0 (±1.2)^{ef}	0.8 (±0.8)^{abc}	0 (±0)^{a}	0 (±0)^{a}	17.2 (±0.8)^{i}	18.0 (±5.3)^{i}
Conostylis neocymosa^{2}	Shelf	0 (±0)^{a}		2.5 (±1.5)^{a}		34.4 (±9.4)^{bcd}	3.0 (±0.6)^{a}	0 (±0)^{a}	28.5 (±3.3)^{bc}	68.0 (±4.7)^{g}
	Shelf+smoke	65.1 (±3.8)^{gf}		52.5 (±8.2)^{gf}		49.6 (±5.1)^{cdef}	37.0 (±8.5)^{bcde}	85.1 (±2.6)^{h}	39.0 (±6.1)^{bcde}	66.5 (±11.0)^{g}
	Buried	1.0 (±1.0)^{a}		0.6 (±0.6)^{a}		29.3 (±4.7)^{bc}	1.0 (±1.0)^{a}	0 (±0)^{a}	38.3 (±5.6)^{bcde}	52.3 (±1.9)^{efg}
	Buried+smoke	43.0 (±5.7)^{cde}		63.8 (±12.0)^{gf}		37.5 (±6.6)^{a}	24.8 (±4.6)^{b}	34.6 (±2.7)^{bcd}	60.2 (±5.9)^{gf}	75.0 (±7.8)^{h}
Stylidium affine^{1}	Shelf	0 (±0)^{a}	0 (±0)^{a}	2.7 (±2.7)^{abc}	0.7 (±0.7)^{ab}	4.1 (±1.2)^{cdef}	1.3 (±1.3)^{abc}	0 (±0)^{a}	1.3 (±0.7)^{abc}	0 (±0)^{a}
	Shelf+smoke	2.1 (±1.2)^{abc}	0 (±0)^{a}	6.8 (±0.7)^{def}	11.5 (±1.3)^{fg}	18.4 (±4.7)^{g}	18.0 (±4.2)^{g}	2.0 (±2.0)^{abc}	0.7 (±0.7)^{ab}	8.1 (±3.5)^{def}
	Buried	0 (±0)^{a}	0 (±0)^{a}	0.7(±0.7)^{ab}	2.0 (±1.1)^{abc}	5.4 (±2.7)^{bcde}	0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}	4.7 (±0.7)^{cdef}
	Buried+smoke	1.4 (±0.7)^{abc}	2.7 (±0.7)^{bc}	10.5 (±2.1)^{efg}	34.4 (±10.7)^{h}	50.3 (±11.0)^{i}	8.3 (±3.2)^{def}	0 (±0)^{a}	0 (±0)^{a}	9.3 (±4.4)^{defg}
Stylidium crossocephalum^{2}	Shelf	0 (±0)^{a}		*		7.2 (±3.5)^{b}	12.8 (±3.1)^{bcde}	0 (±0)^{a}	10.5 (±2.3)^{bc}	34.4 (±1.3)^{fghi}
	Shelf+smoke	29.0 (±7.5)^{fgh}		*		39.8 (±6.2)^{hi}	46.8 (±3.8)^{i}	22.8 (±5.2)^{bdef}	25.2 (±5.4)^{defg}	34.9 (±4.9)^{ghi}
	Buried	0 (±0)^{a}		*		13.3 (±4.7)^{bcd}	0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}	27.2 (±9.1)^{efg}
	Buried+smoke	31.4 (±4.8)^{fghi}		*		41.1 (±6.6)^{hi}	24.6 (±1.5)^{efg}	0 (±0)^{a}	7.6 (±2.4)^{bc}	88.1 (±15.7)^{j}
Deeply dormant										
Conospermum triplinervium^{2}	Shelf	1.6 (±1.6)^{a}		*		1.7 (±1.7)^{a}	0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}
	Shelf+smoke	2.4 (±1.5)^{ab}		*		1.7 (±1.7)^{a}	4.5 (±2.9)^{b}	0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}
	Buried	1.6 (±1.6)^{a}		*		10.7 (±6.2)^{b}	0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}
	Buried+smoke	0 (±0)^{a}		*		0 (±0)^{a}	3.3 (±3.3)^{a}	0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}
Hibbertia commutata^{2}	Shelf	0 (±0)^{a}		0 (±0)^{a}		0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}	
	Shelf+smoke	0 (±0)^{a}		0 (±0)^{a}		0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}	
	Buried	0 (±0)^{a}		0 (±0)^{a}		0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}	2.1 (±1.2)^{b}	
	Buried+smoke	0 (±0)^{a}		0 (±0)^{a}		1.4 (±0.7)^{b}	0 (±0)^{a}	2.7 (±1.8)^{b}	5.6 (±0.7)^{b}	
Stirlingia latifolia^{1}	Shelf	1.8 (±1.8)^{a}	0 (±0)^{a}	0 (±0)^{a}	*	3.6 (±3.6)^{a}	0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}
	Shelf+smoke	7.3 (±4.8)^{ab}	5.2 (±5.2)^{ab}	8.0 (±4.0)^{ab}	*	0 (±0)^{a}	12.1 (±6.0)^{ab}	5.6 (±2.8)^{ab}	3.4 (±3.4)^{a}	3.1 (±3.1)^{a}
	Buried	0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}	*	0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}	33.3 (±33.3)^{b}
	Buried+smoke	1.4 (±1.4)^{a}	0 (±0)^{a}	4.8 (±4.8)^{a}	*	0 (±0)^{a}	16.7 (±16.7)^{ab}	7.7 (±7.7)^{ab}	9.1 (±9.1)^{ab}	16.7 (±16.7)^{ab}
Leucopogon conostephioides^{2}	Shelf	0 (±0)^{a}		0 (±0)^{a}		*	0 (±0)^{a}	0 (±0)^{a}	0.9 (±0.9)^{a}	0 (±0)^{a}
	Shelf+smoke	0 (±0)^{a}		0 (±0)^{a}		*	0 (±0)^{a}	0 (±0)^{a}	0.9 (±0.9)^{a}	0 (±0)^{a}
	Buried	0 (±0)^{a}		0 (±0)^{a}		*	0 (±0)^{a}	0 (±0)^{a}	0 (±0)^{a}	9.5 (±2.4)^{b}
	Buried+smoke	0 (±0)^{a}		0 (±0)^{a}		*	0 (±0)^{a}	0 (±0)^{a}	2.4 (±2.4)^{a}	26.2 (±13.3)^{b}

Data are mean percentage germination (± SE) and values followed by the same letter(s) are not significantly different ($P < 0.05$).

* Omitted due to inadequate seed numbers.

[1] Species sampled four times over the summer season.

[2] Species sampled twice over the summer season.

germination in both dormant and deeply dormant species in response to burial. These patterns of dormancy release were independent of status of dormancy (dormant versus deeply dormant). In addition to the influence of soil burial, dormant species (*C. neocymosa* and *S. crossocephalum*) were also influenced by a possible 'biological clock-like' mechanism.

Dormancy relief occurred after the first (*S. affine* and *C. triplinervium*), second (*A. manglesii, L. conostephioides, H. commutata* and *S. latifolia*) or both summers (*C. neocymosa* and *S. crossocephalum*) and was independent of dormancy status. Summer-induced dormancy relief ensures that germinants appear in autumn, when ambient conditions in the mediterranean-type ecosystems of south-western Australian are conducive to seedling growth and establishment (Fig. 1), rather than opportunistic germination following unsustainable soil wetting which may occur following sporadic and unseasonal summer rainfall events (Karssen, 1980/81; Bell *et al*., 1995). Seeds may subsequently return to a dormant state (secondary dormancy) after autumn, losing their sensitivity to external cues, and avoid germinating at times when germination, seedling establishment and long-term survival are problematic, as noted previously for kwongan species (Roche *et al*., 1998).

Burial was not required for dormancy release for all species tested in this study. Specifically, germination of buried seed of *A. manglesii* and *C. neocymosa* was not higher than that of shelf-stored seeds, either with or without smoke. Hence for these species, after-ripening appears to operate before seeds become receptive to the smoke cue. In contrast, the interaction of burial and response to seasonality dictated germination patterns over the year for other species. Burial for 3, 6 months and 15 months for *C. triplinervium*, *S. affine* and *S. crossocephalum*, respectively, elicited higher germination than in shelf-stored seeds with the addition of smoke. In addition, buried seeds of *H. commutata* germinated at higher levels with smoke treatment. In these species, enhanced germination coincided with either the passing of the first and/or second summer, indicating a strong requirement for soil burial.

The requirement for long-term soil storage has been reported for other species in other ecosystems, including mediterranean-type ecosystems. These include species from the *Leguminosae* (van Staden *et al*., 1994), the *Papaveraceae*, *Lamiaceae* (Keeley and Fotheringham, 1998), *Restionaceae* (Meney *et al*., 1994) and *Epacridaceae* (Roche *et al*., 1997, 1998). For *Dicrostachys cinerea* (*Leguminosae*), a non-mediterranean species, the mechanism of dormancy relief was attributed to a breakdown or weathering of resistant seed coat structures in concert with seasonal fluctuations and/or microbial action, which resulted in improved water permeability (van Staden *et al*., 1994). A similar mechanism may operate following soil burial in *L. conostephioides*, resulting in splitting of the locules comprising the woody fruit (Tieu, 2000). However, an additional external cue in the form of plant-derived smoke was required for germination in *D. cinerea* (van Staden *et al*., 1994) and *L. conostephioides* (Roche *et al*., 1997), and for the latter the mechanism of this process is unknown.

As a corollary, the responsiveness of seeds to smoke was not absolute. Instead, it was a dynamic process and highly dependent on seed age and intrinsic factors such as after-ripening. For example, not all smoke-responsive species germinated immediately at high levels following application of smoke (e.g. *A. manglesii*), nor was smoke responsiveness maintained at a constant level year-round (e.g. *S. crossocephalum* and *C. neocymosa*). In addition, the variation in response to smoke in *A. manglesii*, *C. neocymosa* and *S. crossocephalum* at different times of the year was largely dependent on seed age and not other external factors, such as low rainfall in the preceding year as suggested by Roche *et al*. (1998) for other species from the same genera used in this study. These findings reinforce the importance of smoke as a germination cue (Dixon *et al*., 1995) that allows the full potential for seed germination to be expressed, particularly in *S. affine* and *S. crossocephalum*. In *L. conostephioides*, however, low germination in response to smoke treatment may be attributed to the requirement for microbial, bacterial interactions or a longer period of burial.

Seeds of *S. crossocephalum* and *C. neocymosa* appear to demonstrate control by a biological clock-type mechanism, whereby seeds appear capable of measuring the timing of a season and germinate correspondingly. In these species, germinability appeared to be internally 'pre-programmed' to correspond to seasonal changes without direct effects of environmental cues such as temperature, humidity or light. Regardless of soil burial or shelf storage, non-smoke-treated seeds demonstrated a bimodal germination pattern, which was largely mirrored by slightly elevated germination levels for smoked seeds. Similar results have also been observed in *Poa trivialis* (Froud-Williams *et al*., 1986), *Mesembryanthemum nodiflorum* (Gutterman, 1980/81) and *Picea sitchensis* (Jones *et al*., 1998), and for the Australian native sedge species, *Schoenoplectus validus* (Meney, unpublished). However, the nature and extent of this phenomenon requires further investigation.

The outcomes of this study have important implications for developing methods of understanding and relieving deep dormancy in seeds of native species used for horticulture and landscape restoration. Not all species tested required burial and smoke for high levels of germination. In the context of ecological practice for horticulture and land rehabilitation, high germination levels of *A. manglesii* could be achieved by ensuring that after-ripening was complete, whereas the other test species would require a period of burial followed by smoke treatment. However, *L. conostephioides* does not appear to require smoke treatment, as burial alone may be sufficient to elevate germination. This study also emphasized the need for further empirical research into after-ripening processes for other Australian species, particularly for those taxa of interest in horticulture and land reclamation.

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